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INSECTICIDE INVESTIGATIONS BRANCH

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Fly Control Studies (Laboratory)

Results of laboratory investigations on residual sprays for the control of adult houseflies are based upon a standardized technique which involves the exposure of four day old adult flies of both sexes for 15, 30 or 60 minutes in exposure chambers whose walls are made of panels. treated with various insecticides. The immediate knockdown at the end of each exposure and the resultant 24 hour mortalities are used as criteria of spray evaluation.

It has been found that three day old flies are the most resistant and that the male adults are more susceptible to DDT than the females. In general the figures used are those of the female mortalities which are lower than comparable male kills.

Preliminary observations indicated that while the flies were more susceptible to DDT deposits than adult mosquitoes still the residual deposits appeared to lose effectiveness more rapidly in tests using houseflies than in comparable tests with A. quadrimaculatus mosquitoes. To determine if this effect was a result of actual removal of the deposit by the flies three sets of panels were treated with 25 mg. DDT per sq. ft. and tested as follows: (a) panels exposed to adult flies 1 day after spray application and then only after 14 days, (b) and (c) panels exposed at 1, 6, 7, 8, 9, 13, 14 and 15 days after spray application. In this experiment the (a) set received only 12 batches of flies, while the (b) and (c) sets received 66 batches of flies. Results were as follows, (table 1).

Table 1. Twenty-four hour mortalities (percent) of adult female M. domestica after 15 and 30 minute exposure to 25 mg. DDT per sq. ft. deposits on plywood surfaces.

Panel Age (Days)	Exposure Period (Minutes) 15			30		
	A	B	C	A	B	C
1	1	0	1	4	7	6
6	-	-	-	-	98	100
7	-	30	77	-	88	94
9	-	6	22	-	55	91
13	-	0	0	-	6	41
14	-	2	0	-	9	41
15	97	0	0	98	3	23

All sets of panels gave a very low initial kill and this may have resulted from solvent repellency at a short interval after spray application. These experiments showed that repeated exposure to flies caused a rapid loss of residual toxicity of light DDT deposits. To preclude the possibility that the testing technique might have introduced some factors toward the loss of residual toxicity the experiment was repeated as before with the exception that a fourth set of panels (d) was used. This set was carried through all the mechanical actions of testing with the exception that no flies were placed in the chamber. Results were as shown in table 2.

Table 2. Twenty-four hour mortalities (percent) of adult female, M. domestica after 15 and 30 minute exposure to 25 mg. DDT per sq. ft. deposits on plywood surfaces.

Panel Age (Days)	Exposure Period (Minutes)							
	15				30			
	A	B	C	D	A	B	C	D
8	39	81	48	-	59	93	82	-
9	-	58	48	-	-	78	81	-
14	-	34	18	-	-	69	47	-
15	-	10	15	-	-	75	68	-
21	100	2	4	93	97	60	80	100
49	-	4	1	-	-	74	13	-
50	-	3	2	-	-	54	11	-
52	97	3	1	98	99	19	2	99

This series indicated that the flies were the important factor in the loss of residual toxicity as the (d) series was comparable with the (a) series and had been subjected to all mechanical measures in the technique.

Although the original DDT deposit was light (25 mg. per sq. ft.) and the concentration of flies at each exposure was heavy (50-70 flies per sq. ft. of surface) this experiment indicates that results previously obtained using adult mosquitoes as the test insect cannot be used to directly predict results as to the efficiency of DDT for housefly control.

In field applications to dairy barns for housefly control it has been the practice to use 2½ percent DDT emulsions rather than 5 percent emulsions and to apply a larger volume of spray to obtain the 200 mg. DDT per sq. ft. deposit desired especially on absorbent surfaces. This practice was based on the theory that on absorbent surfaces much of the DDT spray penetrated below the outer surface and was lost as far as residual efficiency was concerned. By using more liquid and a lower concentration of DDT it was thought that relatively less DDT would be lost in this manner. To check this theory in the laboratory the following sets of panels consisting of plywood, rough wood and paper surfaces were sprayed with 2½, 5 and 10 percent DDT-xylene emulsions to give comparable residues of 200 mg. DDT per sq. ft. The paper surface was made up of five layers of standard wallpaper, the rough wood consisted of undressed pine, and the plywood was standard ¼" pine wood three-ply. During the first two weeks all panels were tested without any surface manipulation. Then the "A" set was left unchanged, while the "B" and "C" sets were changed as follows: plywood surfaces had approximately .01" of surface removed, rough wood was treated in a similar manner, and the top layer of the paper was removed. The results of these tests are given in table 3.

These results indicate that (1) the residual effectiveness of the 200 mg. DDT per sq. ft. deposits was not influenced by change in the emulsion, at least within the range of 2½ to 10 percent DDT; (2) only a negligible amount of the DDT penetrated to .01 inch in the plywood or through one thickness of wallpaper but that an appreciable amount penetrated the rough wood; (3) there is evidently a loss of DDT from the wallpaper surface under these test conditions as shown by the decreased effectiveness of the unmanipulated surfaces.

Fly Control Studies (Field)

Houseflies

Field investigations have been conducted to determine the following points in the control of houseflies in dairy barns: (a) the relative value

Table 3. Twenty-four hour mortalities (percent) of adult female, M. domestica, after 30 minutes exposure to 200 mg. DDT per sq. ft. deposits from applications of 2½, 5 and 10 percent DDT-xylene emulsions on plywood, rough wood and wallpapered surfaces.

Type Surface	Panel Age (Days)	Type of Emulsion Used								
		2½ percent			5 percent			10 percent		
		A	B	C	A	B	C	A	B	C
Pine Ply-wood	7	100	98	100	98	99	98	99	99	100
	14	100	97	96	100	99	99	100	99	99
	28	98	1	1	99	1	0	100	0	0
Rough Wood	7	100	100	-	98	100	-	97	100	-
	14	97	98	-	100	100	-	100	99	-
	28	99	55	-	98	2	-	95	53	-
Wall-paper	7	99	100	97	98	100	100	97	100	100
	14	86	93	90	72	97	98	75	85	92
	28	74	0	1	49	1	1	44	0	1

Note: Surface manipulation was performed at 21 day (panel age).

of fall applications of DDT in preventing spring build-up of fly populations, (b) the value of whitewash-DDT applications for fly control, and (c) the value of partial or spot treatments in comparison to complete residual applications. Preliminary work has been done on the evaluation of the toxicant "1068" for dairy applications.

To determine the effectiveness of DDT residues in retarding the spring build-up of houseflies three dairies were treated during the fall of 1945 with the following results: In one dairy after an initial coat of whitewash, a 2½ percent DDT-xylene-Triton X-100 emulsion was applied on September 13th, to give a 200 mg. DDT per sq. ft. residue. During 1946 prior to July 1, the housefly population has been consistently above a weekly grill count index of 10 flies, the established maximum level for satisfactory control.

A second dairy was given a complete premise treatment consisting of milking barn and several outbuildings sprayed with $2\frac{1}{2}$ percent DDT emulsion again at 200 mg. DDT per sq. ft. This application was made in early October and through June only three of the eleven weekly counts have given indices above 10 flies by the grill method.

A third dairy was given a similar application with the exception that only the milking barn was treated. Of the eleven weekly readings this spring only three have been above an index of 10 flies while another three have closely approached this figure.

In the three foregoing dairies' averages of 17.9, 9.6, and 9.8 flies for the weekly spring indices show appreciable reduction as compared to averages of 24.8, 13.0, and 14.5 flies respectively during the same period in non-treated dairies of similar size and sanitary conditions.

Tests on the residual effectiveness of DDT incorporated in whitewash were made at two dairy barns to determine the possible destruction of DDT by whitewash lime and the extent to which DDT crystals were available to contact by flies.

For one test, 35 percent DDT-xylene-Triton concentrate was added to the whitewash so as to constitute a 400 mg. DDT per sq. ft. application. The whitewash-DDT mixture was applied with a power sprayer at 50 psi using a 50 degree fan-shaped spray nozzle delivering 0.6 gals. per minute. An average of 24.8 houseflies was obtained in six weekly pretreatment counts (April 10 - May 20) while five weekly posttreatment counts averaged 4.9 flies.

In a second test a similar treatment was made except that 50 percent DDT water-dispersible powder was used in the whitewash. In this dairy the average of six pretreatment indices was 13.2 flies while the average of five posttreatment indices was 4.2 flies.

In two dairies, in which the sanitation was considered good, only the ceilings of the milking barns were treated to determine the extent of control which might be obtained from partial applications of residual sprays. At the first dairy the ceiling was treated with a $1\frac{1}{4}$ percent DDT-xylene emulsion at the rate of 100 mg. DDT per sq. ft. An average of six weekly pretreatment indices of 24.7 flies was reduced after treatment to a five week average of 4.4 flies.

In the second dairy, $2\frac{1}{2}$ percent DDT-xylene emulsion was used at 200 mg. DDT per sq. ft. and a six week pretreatment average of 20.5 flies was reduced to an average of 2.6 flies for five weeks.

In two dairies treatments of the milking barns were made to determine the residual effectiveness of the spray material "1068" on the housefly. A xylene-Triton emulsion containing $2\frac{1}{2}$ percent "1068" was applied at the rate of 200 mg. "1068" per sq. ft. In one dairy a three week pretreatment average of 68.4 flies for weekly indices was reduced to 3.1 flies for four posttreatment inspections. In the other dairy a pretreatment average of 15.8 flies was reduced to a five week average of 9.1 flies. In this dairy, two pretreatment counts were taken prior to its use for milking cows although dry cows had been pastured nearby for some time.

Blowflies

The value of treating the vegetation immediately adjoining the premise to be protected from blowflies is being compared to more extensive types of application. The comparative values of DDT-emulsions and water-dispersible DDT applications are being considered.

At a seafood plant, 50 percent DDT water-dispersible material was applied to the grasses, bushes and trees within 30 ft. of the oyster and crab picking buildings. A $2\frac{1}{2}$ percent DDT suspension was applied by means of a Bean

50 gal. power sprayer at a pressure of 200 psi. Prior to treatment an average of four weekly blowfly indices was 57.1 and subsequent to treatment the average for five weekly indices was 24.4 blowflies

At a large establishment where green hides were cured before shipment to a tannery a xylene-Triton emulsion containing 2½ percent DDT was applied to vegetation within 30 ft. of the building. In addition, brickwork around the entryways and the interior of the building itself were also treated. Although blowflies usually do not remain within a building overnight, it was considered that the added application was needed as many blowflies were enclosed in the building at the end of each day's work. An average of 238.2 blowflies for nine pretreatment weekly indices was reduced to 48.3 blowflies for three posttreatment inspections.

At a second, much smaller hide plant, the owner had been unsuccessful in controlling blowflies with water-dispersible DDT applied to the building. Treatment of the exterior of the building and the vegetation for 25 ft. around the building with 2½ percent DDT suspension of 50 percent water-dispersible DDT reduced a four week pretreatment average index of 64.1 blowflies to 10.5 blowflies for a three week posttreatment average.

At a farm where chickens were killed twice a week, the interior of the killing house, the entrances and the vegetation within 25 feet of the building were treated with 2½ percent DDT-xylene emulsion to control both house and blowflies. Four weeks prior to treatment the average weekly indices were 11.1 houseflies and 40.1 blowflies. In five weeks after treatment the indices were reduced to 2.9 houseflies and 5.9 blowflies.

In a small woodland pig corral, crab shells were dumped twice weekly and large numbers of fly larvae were found developing. The lower portions of five trees within 30 ft. of the crab shell pile were treated with 2½ percent "1068"-

xylene-Triton emulsion. In six weekly pretreatment inspections the average weekly index was 408 blowflies while in five weekly posttreatment periods the average was 38.3 blowflies.

For the control of blowflies at abattoirs, $2\frac{1}{2}$ percent DDT emulsion and $1\frac{1}{4}$ percent water-dispersible DDT suspensions were tried. At one abattoir the exterior walls of the killing building and the vegetation for 30 ft. were treated with the emulsion. At this establishment an average of 221.5 blowflies for the pretreatment indices was reduced to 52.8 flies for a six week period despite the fact that the grasses and bushes around the abattoir were cut down three weeks after the application and thus reduced the treated area by one half.

At another large abattoir, a $1\frac{1}{4}$ percent DDT suspension of 50 percent water-dispersible DDT was applied at 200 psi to vegetation within 30 feet of a waste tank, to the interior of a building around the tank and to the interior of a partially open tankage room adjoining. A 227 blowfly average for seven pretreatment weekly indices was reduced to 44.5 blowflies for three posttreatment weekly indices.

Mosquito Control Studies (Field)

Work in the field has been directed toward the comparative evaluation of different types of premise treatment for residual control of mosquitoes, and toward determining the comparative explosibility of certain DDT-solvent mixtures.

In order to determine how complete a residual spray job is necessary for control of <u>Anopheles quadrimaculatus</u> in occupied houses a series of nine rooms was furnished with simulated furniture and treated with 5 percent DDT-xylene-Triton X-100 emulsion spray at the rate of 200 mg. DDT per sq. ft.

Three types of application were tried and three rooms were used for each type treatment. The type treatments used were designated as (1) regular treatment as recommended at present in the extended malaria control program consisting of application to the walls and ceiling with the furniture covered in the center of the room, (2) complete treatment which consisted of the regular application plus additional treatment of the undersides and backs of all furniture and pictures, and (3) spot treatment which consisted of spray spplication only to the undersides and backs of furniture and pictures and the wall area immediately behind them plus application in the corners of the room and other likely mosquito resting places. During the period covered by this report data has been obtained from one release of adult mosquitoes in each of these rooms.

In preparation of the rooms for releases, all crevices and cracks were blocked with strips of masking tape, the floors were covered with paper to facilitate recovery of knocked-down adult mosquitoes and the windows and shades were raised during the tests. In making the tests the knocked-down mosquitoes were picked up from the floor and window sill at 15 minute intervals for a period of 4 hours. The data obtained from releases in rooms of one type treatment were handled as duplicate and averaged to give the knockdown percentage per interval.

The results shown (table 4) give the total percentage of female mosquitoes knocked down at the end of each hour, the total number removed from the screens and the total number remaining at the end of the four hour test period.

The following graph shows the comparative fluctuation in knockdown per interval for each type of treatment (figure 1). It appears characteristic of curves showing the most effective treatment that a rather sharp peak is reached early in the test indicating a quick knockdown of a high percentage

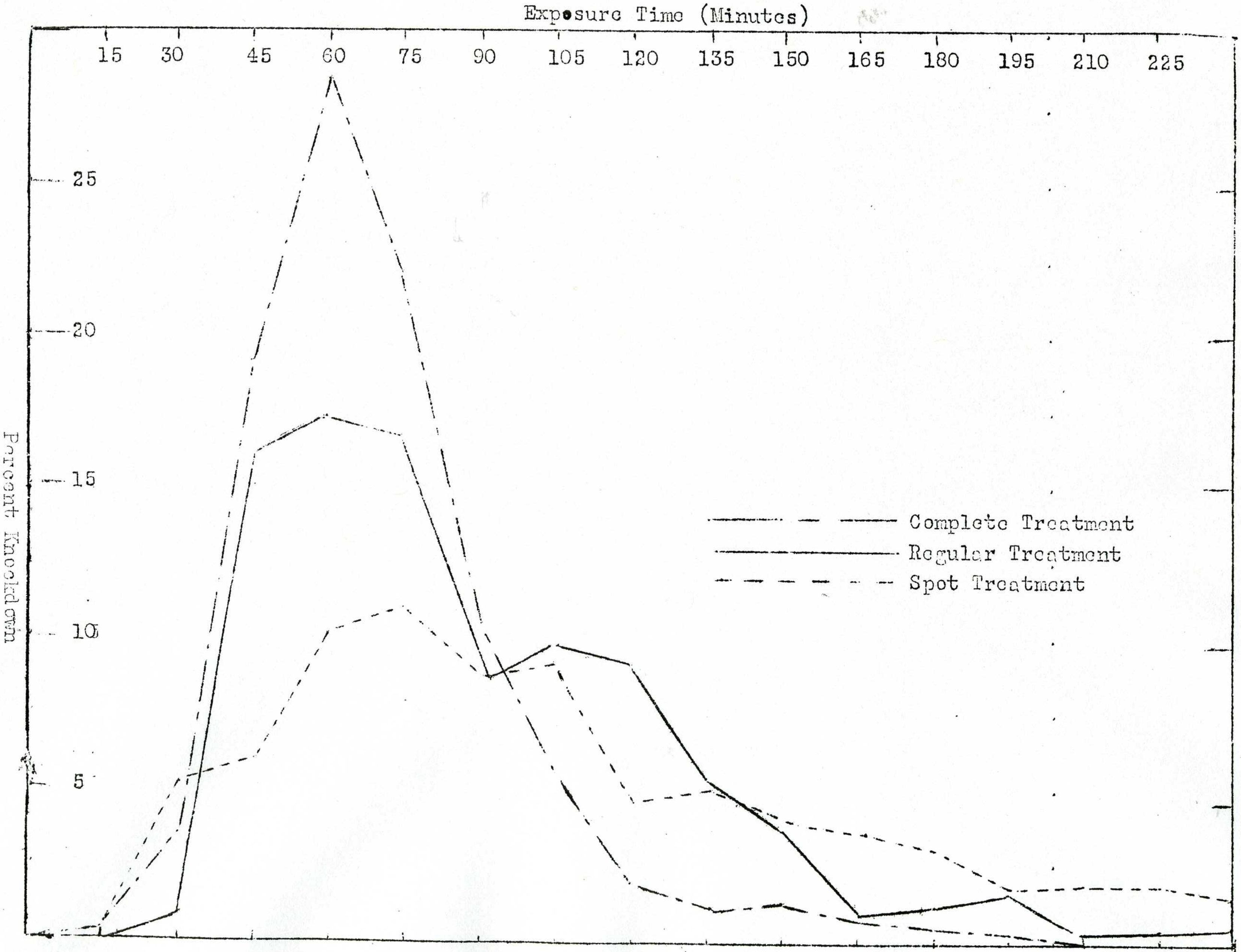

Figure 1. Percent of adult female *Anopheles quadrimaculatus* mosquitoes knocked down at successive 15 minute intervals during exposures to 200 mg. DDT per sq. ft. residues in simulated occupancy rooms.

Table 4. Percent of released female mosquitoes knocked-down after 1, 2, 3, and 4 hours exposures to 200 mg. DDT per sq. ft. deposits 2 months after spray application to different portions of simulated occupancy rooms.

Exposure Period (Hours)	Spot Treatment	Regular Treatment	Complete Treatment
1	21.69	34.33	51.57
2	55.39	78.95	91.94
3	71.19	89.95	95.54
4	78.32	92.63	95.86
Total on screens	8.32	6.77	4.25
Still up	14.1	.74	0.0

of mosquitoes in comparison to less effective treatments with lower peaks occuring later.

Although these data are merely preliminary it is believed that certain trends are indicated. The rate of knockdown appears to vary in direct proportion to the amount of surface treated as shown by the wide divergence between the spot job and the complete job results. It required four hours for the spot application to produce a total knockdown approximately equal to that produced by the regular application in two hours. At the end of two hours the knockdown from the regular application was significantly less than from the complete coverage. As shown in the table both the regular and complete jobs were superior to the spot job.

The number of mosquitoes found on the screens in these tests varies inversely with the effectiveness of the treatment. It is difficult to explain this in view of observations by others that DDT-affected mosquitoes seek the light. It would seem that the more insects that were affected the more would be found on screens. It is likely that this tendency is counteracted by mosquitoes receiving heavier doses of DDT in the more completely treated rooms thus causing many to be knocked down too quickly to be counted on the screens.

As an initial check mosquitoes were released in an untreated room with simulated furniture. Only 1 of 245 females was down during the 4 hour period. A sample is taken of each release cage of mosquitoes and held in an untreated room to determine any natural weakness in the daily test mosquitoes.

Comparative explosibility tests were made on DDT-xylene concentrate, DDT-toluene concentrate and DDT-toluene emulsion. The substitution of toluene for xylene as a DDT solvent markedly increases the explosive hazard of the emulsions even at 5 percent DDT concentration.

Insectary

Mosquitoes:

In rearing _A. quadrimaculatus_ mosquitoes for laboratory and field experimental techniques it is necessary to procure daily a constant number of healthy adults. In a study of the general insectary routine it was found that the larval stage had the highest mortality in mosquito production as only 12.6 percent of the eggs set reached the pupal stage. The high larval mortality appeared to be correlated with an excess of decomposing food, larval skins and metabolic waste products. This "sour" condition led to the formation of a film on the water which partially immobilized the mosquito larvae.

To determine whether a change of water in the rearing pans would improve conditions two series of pans were handled as follows: "A" series, referred to as "dirty pans" were run under routine conditions, "B" a second series, "clean pans" had the water changed 8 days after the eggs were set. In changing the water, floating food particles, larval skins and old food were poured off and new water and food placed in the pans. Results of these experiments (table 5) showed this water changing procedure to be deleterious. It was also noted that a large number of larvae were unaccounted for and this percentage may be attributed to cannibalism. In the "dirty pans" the largest portion of the larval mortality occurred during the first 4 days and the 13th to 15th days after setting the eggs. In contrast, the "clean pans" had the largest larval mortality on the 9th and 10th days after setting the eggs or in the period immediately after the changing of the water. These differences in larval mortality together with the suspected cannibalism indicated a shortage of food during certain stages of the larval development rather than water contamination.

Table 5. Comparable development of A. quadrimaculatus larvae in routine operations and with change of water in the rearing pans.

	"Dirty Pans"	"Clean Pans"
Total 1st instar larvae per series	3719	3785
Total larval mortality	834	910
Total number of pupae	524	451
Total number live larvae on 16th day	303	423
Total number of larvae unaccounted for	2061	2001

In view of the indicated relationship between food supply and larval mortality it was thought that the distribution of the food on the surface of the water might influence its availability to the larvae. Under routine feeding methods powdered brewers yeast was dusted on the surface of the water but there were indications it sank to the bottom of the pans after a short period. To prevent this the yeast powder was placed on wax paper floats and in this manner was prevented from sinking or dispersing and yet was available to the larvae. With this feeding method it was found that the mosquito larvae developed more uniformly and more rapidly through the first two instars but this method did not supply sufficient food for later instar larvae.

Further tests were made using the wax paper floats during the first five days of larval development supplemented with routine dusting of the water with food. With this method of feeding it was found that 26 percent of the larvae reached the pupal stage in contrast to the previous 12 percent under routine feeding.

Houseflies

In jars of culture medium for rearing M. domestica a yeast fermentation line, ½ to 1 inch in depth, forms on the second day of incubation. This line occurs about 2½ inches below the surface of the culture medium, where the temperature of 48.8° C. is most favorable to fermentation. In an effort to conserve culture medium, which is difficult to obtain, jars are filled only to a depth of 4½ inches. The additional 1½ inches allows for settling of medium. Since the larvae will not penetrate below the yeast line the medium below the line is not utilized as larval food.

Sand is added to the fly rearing jars for pupation and 3 to 4 inches may be necessary with relative humidities of 72 percent or over since excess humidity or moisture content of the culture medium will wet the sand and retard pupation, whereas 2 to 2½ inches will suffice at 50-60 percent relative humidity and relatively dry medium.

In the interests of conserving space, it was deemed advisable to study methods of procuring faster production and to determine optimum conditions for larval growth. Under the present system pupae are taken from the rearing jars on the eighth day after setting the eggs. Preliminary studies revealed 99 percent pupation after eight days. A study of seven day old rearing jars revealed only a few larvae and this precipitated the belief that jars seven days of age might be as profitably picked as though eight days old, thus eliminating one day from the cycle. A study was made on jars with 5, 6, 7 and 8 days incubation with the following results, (table 6).

Table 6. Pupation rate under routine insectary conditions.

Age of Jar (Days)	Number of Larvae	Number of Pupae	Total Pupae & Larvae	Percent of Larvae	Percent of Pupae	Average Temp. and Rel. Hum.
5	1243	1181	2424	51.28	48.72	76.5° F.
6	805	3134	3939	20.43	79.57	
7	64	3326	3389	1.88	98.12	64.7 %
8	33	3729	3762	0.87	99.13	

This indicates that the pupae can be picked from jars of 7 days' incubation but that it would not be profitable to pick pupae at an earlier date unless there was an emergency demand.

To determine the effect of increased temperature upon the larval growth and the subsequent rate of pupation several series of jars were placed at increased temperature (90° F.) for 14 hours each day. The results given in table 7 may be compared to those of table 6 as the same numbers of jars were handled in each series.

There have been no indications that the larvae, pupae or resultant adults raised under increased temperature conditions are any smaller or less resistant to the action of insecticides than those raised at the routine temperatures. The experiment indicates that with proper apparatus for maintaining a higher temperature the pupae might be picked at the end of 6 days with little loss of efficiency.

Table 7. Pupation rate under increased temperature.

Age of Jar (Days)	Number of Larvae	Number of Pupae	Total Pupae & Larvae	Percent of Larvae	Percent of Pupae	Average Temp. and Rel. Hum.
5	736	1364	2100	35.00	65.00	83.4° F.
6	212	2469	2681	7.90	92.10	
7	35	2460	2495	1.00	99.00	68.3 %
8	14	2590	2604	0.53	99.47	

ANOPHELINE LARVICIDING

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Dosage - Mortality.

Field tests were conducted to determine the minimum practical dosage of DDT which would give satisfactory control of anopheline larvae when used in No. 2 fuel oil at the rate of one gallon per acre. Dosages tested ranged from 0.5 lb. DDT per acre down to 0.001 lb. per acre. The results were determined by dipping for larvae one day, three days, five days and seven days after treatment. In some few cases the seven day results were not obtained and in most cases the dipping was continued on the tenth and fourteenth days. In all cases the time-response curve was similiar, showing the greatest reduction during the first five days, there being rather little difference between the first, third, and fifth day results. On the seventh day the population was building up again in most cases. Therefore the results for the first, third, and fifth days were averaged together and the results plotted against the dosage of DDT applied. There were four replications of each dosage and the percent control was figured by substracting the ratio of the average posttreatment population to the average pre-treatment population from one and multiplying by one hundred. The results are presented in figure 2, in which the percentage control is plotted against the logarithm of the dosage. Apparently the slope of the curve is rather steep and the control drops off rapidly as the dosage is reduced below 0.05 lb. per acre. Even though appreciable toxicity is evident in lower dosages, it would seem unsafe to recommend less than 0.05 lb. DDT per acre for mosquito control.

In another series of tests certain ponds are being inspected frequently and resprayed with designated dosages as often as necessary to keep the pop-

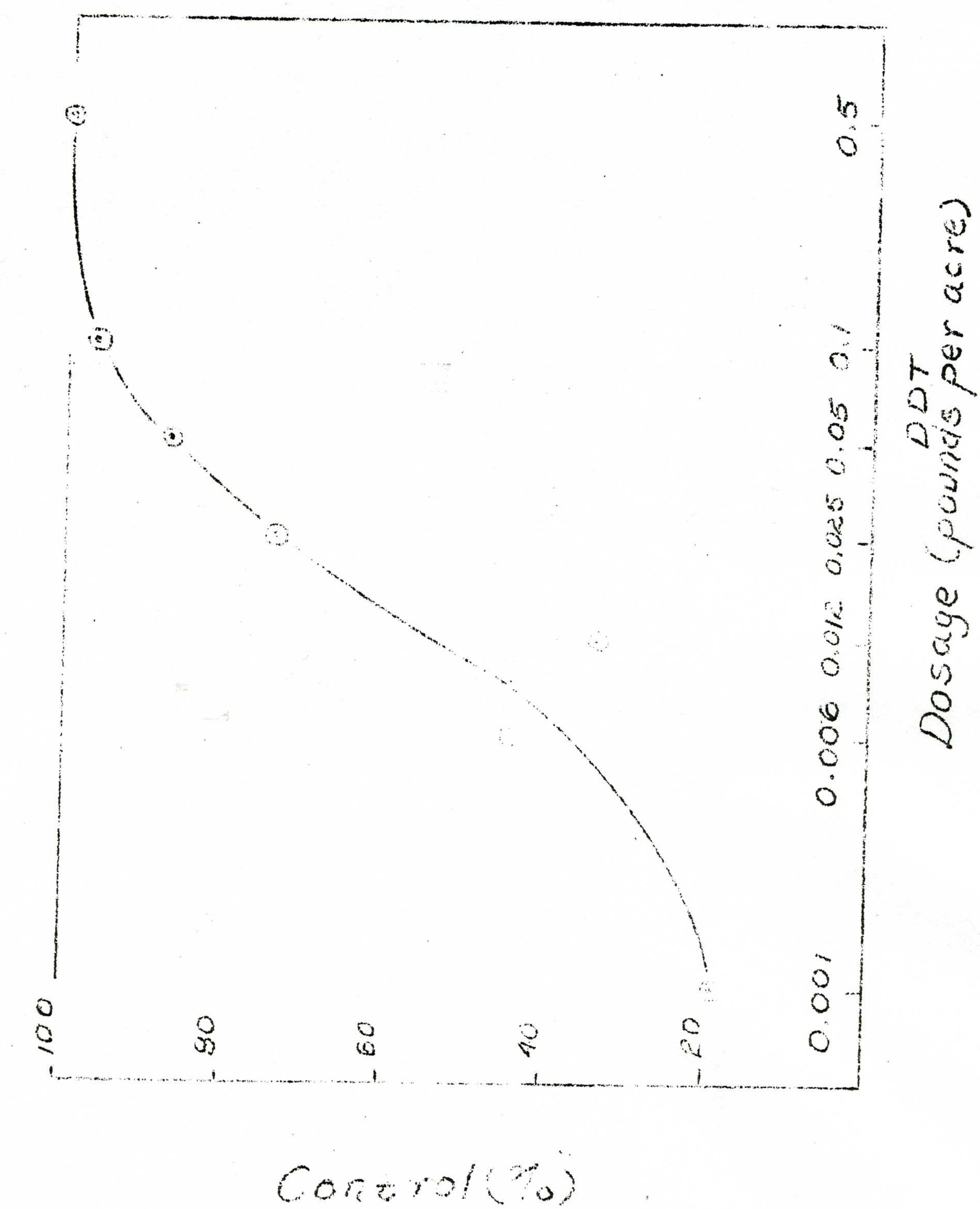

Figure 2. Dosage - response curve for DDT in 1 gal. fuel oil applied as an atomized spray (Dosage expressed on logarithmic scale).

ulation of anopheline larvae low enough so that not more than one dip containing 3rd or 4th instar larvae is found in a series of 30 dips. This series is to be continued throughout the season but early season results confirm the above dosage - mortality data, indicating that unless at least 0.05 lb, of DDT is used per acre, the first treatment may not produce satisfactory control.

A similar dosage - mortality series using various volumes of fuel oil without added DDT or spreader, indicated that it is possible to obtain appreciable anopheline larval control (85 percent over the first five days) with as little as 10 gallons of fuel oil per acre. As the dosage was reduced below this, the effectiveness fell off very rapidly until one gallon per acre produced no detectable control.

DDD.

Preliminary tests using dichloro diphenyl dichloroethane (DDD; also known as TDE) in one gallon of fuel oil using dosages of 0.05, 0.025, 0.006, and 0.001 lb. per acre indicate slightly but consistently higher control than is obtained by the same dosages of DDT.

Table 8. Comparative effectiveness of DDD and DDT in 1 gallon of fuel oil with dosages ranging from 0.001 to 0.05 lbs. per acre.

	1	3	5	7	10	14	Days after treatment
DDD	75.3	80.5	82.3	64.6	56.7	86.6%	reduction from pretreatment
DDT	65.2	78.5	78.2	79.4	45.5	29.5%	reduction from pretreatment

The average results over all dosages are shown in table 8. Essentially similar results were obtained with DDD used as a dust and with DDD used as a spray against culicine larvae found in swamps and borrow pits. These field results substantiate laboratory results previously reported.

Hexachloro cyclohexane.

Previously reported laboratory studies had indicated that the gamma isomer of hexachloro cyclohexane is sufficiently soluble in distilled water to produce high toxicity among insectary-reared larvae of *Anopheles quadrimaculatus*. Further tests with dilutions of the solution indicated its concentration (assuming the gamma isomer to be the only toxic ingredient) to be in the neighborhood of 10 parts per million. On this basis it appeared that application techniques in the field might be greatly simplified. Accordingly a bag consisting of several thicknesses of cheesecloth and containing about one fifth pound of technical "benzene hexachloride" was suspended in each of two infested ponds. No noticeable reduction in larval population was obtained during the first week or longer.

Sabadilla.

A dust concentrate (Sabacide)* containing 50 percent Sabadilla seed was diluted with a kaolin type clay and applied to infested ponds at the rate of one pound of the seed to an acre. An average of 90 percent control of anopheline larvae over the first five days was obtained. The material is somewhat irritating to handle. Further work will be necessary before it can be properly evaluated.

Paraformaldehyde.

In a preliminary field test 10 lbs. of paraformaldehyde per acre applied as a dust produced excellent mortality, no living larvae being found for several days after treatment.

Other materials.

Azobenzene dissolved in fuel oil and applied at a dosage of 0.8 lbs. per acre, and a dust containing 0.16 percent pyrethrins and 2.0 percent

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* A product of the McConnon & Co. of Winnona, Wisconsin.

piperonyl cyclohexanone applied at 10 lbs. per acre gave unsatisfactory results in preliminary fields trials.

EFFECTS OF DDT MOSQUITO LARVICIDING ON WILDLIFE

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Studies on Surface Organisms.

Analysis of samples taken last year and compilation of the results have been actively continued during the past quarter. The results of treatments in ponds 5 and 6, as indicated by quantitative surface samples, are summarized in table 9. These ponds had sand bottoms, were temporary, and resulted from the overflow of the Canoochee River.

Pond 5 was treated with 0.1 pound of DDT per acre in a fuel oil solution to which was added 0.5 percent of a spreading agent. Some significant changes appear to have resulted from the three treatments. There was a significant decrease in total organisms after the first treatment and a general decrease in the mayflies and midges, whereas the copepods, ostracods and nematodes showed a distinct increase after the third treatment.

Emulsions were used for the larviciding in pond 6. The first application consisted of an emulsion made by adding 1 gallon of fuel oil containing 0.1 lb. of DDT and 0.5 percent of a spreading agent to 14 gallons of water. Treatment was at the rate of 15 gallons of emulsion per acre for both the first and second treatments, but the amount of oil and DDT was doubled for the second application. Surface Hemiptera and Coleoptera were killed by both treatments, and there was a marked decrease in the mayflies and chironomids; however, nematodes, oligochaetes and copepods increased to such an extent that there was a significant increase in the total population after each treatment.

Test pond number 7 had a permanent inflow of water from a nearby artesian well. It was given weekly routine treatments at the rate of 0.1 lb. of DDT and 1 gallon of fuel oil per acre. Treatment began early in July and was

Table 9. Changes in the population of surface organisms in two test ponds due to treatments with DDT larvicides as shown by paired square foot samples taken just before and 48 hours after each treatment.

Pond	Pond Number 5								
Treatment and dates	First 5/17/45			Second 5/24/45			Third 6/1/45		
Dosage per acre	1 gal. fuel oil, 0.1 lb. DDT			2 gal. fuel oil, 0.1 lb. DDT			2 gal. fuel oil, 0.1 lb. DDT		
Number of Paired samples	8			7			10		
	Number of Organisms Before	After	Mean difference and its standard error	Number of Organisms Before	After	Mean difference and its standard error	Number of Organisms Before	After	Mean difference and its standard error
Nematoda	65	83	2.3 ± 3.6	77	89	1.7 ± 6.4	72	181	10.9 ± 6.0
Oligochaeta	163	138	- 3.1 ± 7.8	62	357	42.1 ± 21.2	2062	402	-166.0 ± 81.8
Cladocera	184	130	- 6.8 ± 5.9	104	207	14.7 ± 10.7	210	237	2.7 ± 9.2
Copepoda	547	637	11.3 ± 16.8	545	890	49.3 ± 40.1	705	856	115.1 ± 33.5**
Ostracoda	28	17		16	50	4.8 ± 2.7	116	598	48.2 ± 17.4*
Palaemonetes	6	5		4	4		4	15	
Hydracarina	6	6		5	2		11	13	
Ephemeroptera	82	23	- 7.4 ± 3.0*	23	10	- 1.9 ± 0.6*	7	15	
Anisoptera	15	3		2	2		1	2	
Zygoptera	10	4		3	7		8	21	
Hemiptera	6	3		2	2		6	16	
Coleoptera	7	4		4	5		2	3	
Chironomidae	625	68	-69.6 ± 29.9	50	23	- 3.9 ± 2.7	106	27	- 7.9 ± 3.4*
Other Diptera	24	11		5	2		14	7	
Totals	1785	1136	-81.1 ± 31.7*	905	1655	107.1 ± 71.2	3328	3396	6.8 ± 95.6

* Exceeds 5 percent level of significance

** Exceeds 1 percent level of significance

Table 9. Changes in the population of surface organisms in two test ponds due to treatments with DDT larvicides as shown by paired square foot samples taken just before and 48 hours after each treatment. (continued)

Pond	Pond Number 6					
Treatment and dates	First 5/17/45			Second 5/24/45		
Dosage per acre	Emulsion: 14 gal. water, 1 gal. fuel oil, 0.1 lb. DDT			Emulsion: 13 gal. water, 2 gal. fuel oil, 0.2 lbs. DDT		
Number of paired samples	10			4		
	Number of Organisms Before	After	Mean difference and its standard error	Number of Organisms Before	After	Mean difference and its standard error
Nematoda	258	389	13.1 ± 9.8	75	122	11.8 ± 15.8
Oligochaeta	417	634	21.7 ± 18.5	81	109	7.0 ± 24.5
Cladocera	216	310	9.4 ± 4.6	66	75	2.3 ± 12.6
Copepoda	698	1949	125.1 ± 30.1**	806	1029	55.8 ± 35.2
Ostracoda	202	166	- 3.6 ± 16.6	4	39	8.8 ± 2.3*
Palaemonetes	3	8		3	2	
Hydracarina	71	58		30	36	1.5 ± 5.5
Ephemeroptera	65	24	- 4.1 ± 2.1	6	3	
Anisoptera	5	4		0	4	
Zygoptera	4	9		3	16	
Hemiptera	6	5		-	-	
Coleoptera	3	6		0	1	
Chironomidae	329	58	- 27.1 ± 10.1*	22	4	- 4.5 ± 4.5
Other Diptera	22	23		7	2	
Totals	2302	3648	134.6 ± 38.1**	1109	1443	83.5 ± 93.4

* Exceeds 5 percent level of significance

** Exceeds 1 percent level of significance

discontinued in December. The effects of the various individual treatments are summarized in table 10. Gross observations indicated that the first two applications killed a large number of Coleoptera and Hemiptera. Members of these orders were found dead after each of the 22 applications applied to the pond, indicating a reduction but not an elimination of surface forms. Surface sampling was discontinued after the seventeenth treatment. As indicated in table 10, few significant changes occurred due to individual treatments. However, long term or cumulative effects were noted after treatment had continued for a number of weeks. The larger members of the families Gyrinidae, Dytiscidae, Haliplidae, Hydrophilidae, Corixidae and Gerridae became quite scarce after several treatments. Further, the quantitative surface samples indicated a reduction in Chironomidae and Ephemeroptera while there was an increase in Oligochaeta.

All data secured to date tend to confirm and strengthen the conclusions drawn from the data reported heretofore namely: that routine treatment with fuel oil solutions at rates of 0.1 lb. or 0.05 lb. of DDT per acre cause a decrease in the chironomids, mayflies, beetles and water boatmen and an increase in the number of nematodes, oligochaetes and copepods; that the populations of surface insects are kept at a level below their natural abundance by routine treatment; and that oil solutions are more toxic than dusts.

Airplane Applications.

The second season of routine studies was initiated the first week of April. During this season emphasis is placed on an investigation of the effects of airplane application of sprays and aerosols at the rate of 0.1 lb. of DDT per acre. A 20 percent solution of DDT in Velsicol is used for both the spray and aerosol treatment. Four ponds are under treatment; two are being sprayed while the other two receive the aerosol, or exhaust generated

Table 10. Effects on the surface organisms in test pond number 7 of the routine use of 0.1 pound of DDT in 1 gallon of fuel oil per acre as shown by square foot surface samples taken just before and 48 hours after each treatment.

Treatments and dates	First 7/9/45			Second 7/17/45			Fourth 8/1/45		
Number of paired samples	10			10			10		
	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error
Nematoda	17	24		6	11		35	5	- 3.0 ± 2.6
Oligochaeta	22	32	1.0 ± 1.0	47	19	- 2.8 ± 1.4	335	155	- 18.0 ± 20.2
Cladocera	2389	1895	- 49.4 ± 99.1	2028	1770	- 25.8 ± 110.5	1760	2484	72.4 ± 72.8
Copepoda	7587	7051	- 53.6 ± 252.5	6288	5426	- 86.2 ± 165.4	5617	7862	224.5 ± 214.3
Ostracoda	269	252	- 1.7 ± 9.5	349	212	- 13.7 ± 10.0	187	106	- 8.1 ± 3.6*
Hydracarina	23	14	- 0.9 ± 1.0	45	11	- 3.4 ± 2.0	19	23	0.4 ± 2.1
Collembola	2	3		0	4		0	6	
Ephemeroptera	20	10	- 1.0 ± 0.9	26	9	- 1.7 ± 0.9	17	5	- 1.2 ± 1.2
Anisoptera	68	58	- 1.0 ± 1.9	63	50	- 1.3 ± 2.4	100	65	- 3.5 ± 2.7
Zygoptera	8	15		9	10		16	8	- 0.8 ± 0.8
Hemiptera	12	23		9	8		3	7	
Coleoptera	12	12		10	11		4	4	
Chironomidae	4550	1030	-352.0 ± 88.2**	710	104	- 60.6 ± 32.1	607	130	- 47.7 ± 19.9*
Other Diptera	82	46	- 3.6 ± 1.8	17	19		70	10	- 6.0 ± 1.9
Gastropoda	6	3		11	1		13	0	- 1.3 ± 0.5*
Totals	15083	10475	-460.8 ± 400.1	9630	7668	-196.2 ± 299.1	8789	10870	208.1 ± 275.4

* Exceeds 5 percent level of significance

** Exceeds 1 percent level of significance

Table 10. Effects on the surface organisms in test pond number 7 of the routine use of 0.1 pound of DDT in 1 gallon of fuel oil per acre as shown by square foot surface samples taken just before and 48 hours after each treatment. (continued)

Treatments and dates	Sixth 8/14/45			Eighth 8/28/45			Tenth 9/13/45		
Number of paired samples	9			10			10		
	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error
Nematoda	40	148	12.0 ± 9.0		Not Counted		104	157	5.3 ± 8.2
Oligochaeta	430	231	- 22.1 ± 12.5	247	226	- 2.1 ± 10.3	747	432	- 31.5 ± 29.8
Cladocera	2054	130	-213.7 ± 104.9	164	123	- 4.1 ± 4.1	132	205	7.3 ± 7.5
Copepoda	3163	2002	-129.0 ± 95.3	289	422	13.3 ± 6.1	558	1713	115.5 ± 82.9
Ostracoda	287	118	- 18.8 ± 12.1	108	183	7.5 ± 7.7	106	171	0.5 ± 3.9
Hydracarina	56	1	- 6.1 ± 3.4	11	8		8	36	2.8 ± 1.6
Collembola	3	1		12	1	- 1.1 ± 0.7	3	6	
Ephemeroptera	32	2	- 3.3 ± 3.1	1	0		10	7	
Anisoptera	48	13	- 3.9 ± 1.5*	7	10		30	20	
Zygoptera	24	21		6	7		8	14	
Hemiptera	7	3		5	0		4	5	
Coleoptera	8	4		6	3		5	6	
Chironomidae	124	28	- 10.7 ± 4.9	14	4	- 1.0 ± 0.7	407	273	- 13.4 ± 10.5
Other Diptera	30	9	- 2.3 ± 0.9*	36	21	- 1.5 ± 1.4	61	25	- 3.6 ± 2.1
Gastropoda	8	9		9	18		61	34	- 2.7 ± 2.2
Totals	6317	2722	-399.4 ± 201.5	915	1026	11.1 ± 23.7	2307	3105	79.8 ± 121.7

* Exceeds 5 percent level of significance

Table 10. Effects on the surface organisms in test pond number 7 of the routine use of 0.1 pound of DDT in 1 gallon of fuel oil per acre as shown by square foot surface samples taken just before and 48 hours after each treatment. (continued)

Treatments and dates	Eleventh 9/19/45			Thirteenth 10/2/45			Fifteenth 10/15/45		
Number of paired samples	10			10			10		
	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error	Number of Organisms Before	Number of Organisms After	Mean difference and its standard error
Nematoda		Not	Counted		Not	Counted		Not	Counted
Oligochaeta	1239	613	-62.6 ± 49.2	909	711	- 19.8 ± 16.3	962	944	- 1.8 ± 72.8
Cladocera	149	57	- 9.2 ± 7.3	413	587	17.4 ± 12.8	1466	356	-111.0 ± 54.2
Copepoda	1121	1680	55.9 ± 68.7	5856	7338	198.2 ± 242.1	7407	2527	-488.0 ± 169.4*
Ostracoda	146	215	6.9 ± 10.2	532	378	- 15.4 ± 15.7	1084	428	- 65.6 ± 39.2
Hydracarina	24	6	- 1.8 ± 1.2	23	54	3.1 ± 1.5	177	62	- 11.5 ± 8.6
Collembola	23	28		44	153	10.9 ± 4.9	13	10	
Ephemeroptera	3	3		11	10		28	3	- 2.5 ± 2.2
Anisoptera	17	19		105	108		163	68	- 9.5 ± 6.3
Zygoptera	21	11		22	18		18	9	
Hemiptera	1	1		4	6		1	0	
Coleoptera	6	7		3	6		17	6	- 1.1 ± 1.1
Chironomidae	35	46	1.1 ± 1.5	114	112		89	53	- 3.6 ± 2.9
Other Diptera	490	208	-28.2 ± 18.4	215	178	- 3.7 ± 7.4	127	61	- 6.6 ± 5.6
Gastropoda	138	27	-11.1 ± 8.1	46	24	- 2.2 ± 1.7	217	67	- 15.0 ± 8.7
Totals	3418	2922	-49.6 ± 136.2	8298	10183	188.5 ± 259.6	11771	4595	-717.6 ± 324.7

* Exceeds 5 percent level of significance

Table 10. Effects on the surface organisms in test pond number 7 of the routine use of 0.1 pound of DDT in 1 gallon of fuel oil per acre as shown by square foot surface samples taken just before and 48 hours after each treatment. (continued)

Treatments and dates	Seventeenth 10/29/45		
Number of paired samples	10		
	Number of Organisms Before	After	Mean difference and its standard error
Nematoda		Not	Counted
Oligochaeta	1647	659	- 98.8 ± 62.3
Cladocera	761	981	22.0 ± 36.0
Copepoda	4039	3850	- 18.9 ± 99.9
Ostracoda	589	455	- 13.4 ± 10.9
Hydracarina	72	43	- 2.9 ± 1.8
Collembola	3	3	
Ephemeroptera	16	5	
Anisoptera	105	76	- 2.9 ± 3.4
Zygoptera	21	25	
Hemiptera	-	-	
Coleoptera	2	3	
Chironomidae	11	7	
Other Diptera	30	23	
Gastropoda	207	339	13.2 ± 8.5
Totals	7510	6471	-103.9 ± 156.6

sprays. A total of 815 acres are being routinely treated each week while the check or untreated area comprises 850 acres.

Pretreatment studies were initiated in these areas in September 1945. Check and treated areas for studies on mammals, birds, reptiles and insects were selected, mapped, and laid out in grids. Pretreatment studies of the biota were made during the winter and early spring months. One hundred surface sampling stations and 40 bottom sampling stations were selected and staked in each of the 6 areas under study. It is planned to take three series of samples at these stations: the first before treatment, the second at about the midpoint of the treatments and the last after treatment is completed. Fish population studies have been made before treatment and will be repeated at the completion of treatment to determine any effects on the fish population. These studies are being supplemented by gross observations for fish kill after each treatment. Some kills of fish have been noted to date. However, no conclusions can be drawn as yet from the studies of fish, surface and bottom organisms. Gross observations indicate an extensive kill of adult damsel flies.

Insect Studies.

Studies of the insect fauna are being carried on in the marginal zones of all treated areas and in check plots. Collecting trays and observations of dead insects on the water surface are employed to determine the species affected. Light traps are used to take quantitative samples of the insect population in the check plots and in treated areas just before and after each treatment.

To date there have been no discernible changes in the overall insect fauna of the treated areas. Preliminary light trap samples indicate there may be fewer chironomids in the treated areas, but the data thus far are not at all conclusive.

Examination of treated water surfaces shows moderate mortality of dragon flies, damsel flies, aquatic beetles and true flies, but as considerable numbers of these groups are still in evidence in the treated areas, the present total of 8 treatments has certainly not been decimating them.

Preliminary studies of aphid colonies in the treated areas show as many parasites and predators as in the untreated areas. This method is being used to check upon the reported selective killing of aphid predators and parasites.

Five colonies of honey bees in the treated area still are strongly active in the field and are showing a good honey production, so there is, as yet, no indication of any effect upon bees.

Unless there is some accumulative effect of the DDT which produces a drastic change, it is believed the differences in insect populations attributable to DDT, in this season's tests, may be small. What the effect will be in cutting down next year's insect fauna can not be indicated in any way by this season's results, as the adults may be alive and in evidence in good numbers, yet fail to live until they oviposit.

Birds.

The measurement of breeding bird populations at the Savannah River Refuge in relation to the airplane spraying of DDT has been conducted in two ways -- weekly censuses of singing male birds and weekly counts of the species and numbers of birds seen on sprayed and unsprayed areas.

A census of singing males has been long regarded as one of the best ways to keep check on a population of breeding birds. During the nesting season most passerine birds establish territories with the nest as the center of the territory. Within this area they live, feed and rear their young. Each pair of birds protects its territory from other birds of the same species.

The singing of the male acts as a warning to other birds of the same species that the area is occupied. Any cessation of song by a male on an established territory is good evidence that the bird or its mate has been killed in one way or another. By taking censuses 24 hours after each weekly spraying, one is able to evaluate the influence, if any, of DDT on the birds.

The bird census areas at the Savannah Refuge consist of ten small islands and nine dykes. The dyke areas vary in length, but the width of each is 200 feet -- 100 feet out into the marsh on each side of the dyke.

Six islands (18.91 acres) and five dykes (27.11 acres), serving as experimental areas, are sprayed weekly when the ponds adjacent to them are sprayed. The other four islands (10.63 acres) and four dykes (20.21 acres) serve as check areas. Individual maps of the islands and dykes are used for each census, and the locations of singing males are plotted on the maps. All birds seen and heard during the census are enumerated, according to species, on the back of the map.

At monthly intervals the locations of singing males recorded on each island and dyke during the weekly censuses are consolidated on individual maps -- one map for each species heard on each island and dyke. The total number of individual birds of each species seen on the islands and dykes are also consolidated.

By studying these consolidations of singing records one is able to determine the extent of territory of various pairs, the dates on which they were singing and hence the presence or absence of males on their territories. The consolidations of total numbers of birds seen and heard on the weekly censuses also help to determine the extent of population fluctuations.

The first census of singing males was made on March 15 when about half of the islands and dykes were covered. Between March 26 and April 2, the remainder of the islands and dykes (dyke 1 excepted) were censused.

The following information covers the period from March 15 to May 30 for the area shown as pond 6. A total of 47 singing males of 20 species was recorded on the 10.61 acres of the unsprayed inslands: I, II, IX, and X. Thirty-five singing males were recorded on these islands up to April 30, the day before spraying began. On the unsprayed dykes A, B and C, consisting of 11.49 acres, 29 singing males of 8 species were heard. Twenty-five singing males were heard on these dykes up to April 30.

A total of 68 singing males of 23 species was recorded on the 18.91 acres of the sprayed inslands: III, IV, V, VI, VII and VIII. Sixty-three singing males were recorded on these islands up to April 30. On the sprayed dykes, D, E, and F, consisting of 9.83 acres, 17 singing males of 8 species were heard. Thirteen singing males were heard on these dykes up to April 30.

The increase of singing males after May 1st was caused by the arrival of late migrants. The four sprayings during May on islands III, IV, V, VI, VII and VIII and dykes D, E, and F, had no apparent effect on the males that had been singing in March and April, for their songs continued in May. Likewise the sprayings had no apparent effect on the total numbers of birds counted on the islands and the dykes before and after spraying.

Rat and Mouse Population Studies.

One method of keeping a check on the rat population of an area is to live-trap, mark, release, and retrap the animals. This is being done on sprayed and unsprayed areas at the Savannah River Refuge.

From April 30 to June 28, 50 live traps were used over a period of 1778 trap-nights. During this time 86 cotton rats, 3 rice rats, 5 wood rats, 2 cotton mice, and 18 house mice were trapped alive, tagged in the ears with numbered Monel metal fingerling tags and released. Twenty-nine of these animals were trapped in sprayed areas and 35 in unsprayed areas. Since only

50 live-traps are available for this work, it is necessary to rotate them from week to week among the various islands and dykes. Thus an area is retrapped once about every third week.

To date 15 animals have been retrapped once, 2 twice, and 3 three times. Ten of the retrapped animals were taken in sprayed areas and seven in unsprayed areas. Rat populations seem to have been holding up rather uniformly, and to date there has been no evidence of DDT poisoning or death due to this compound.

Observations on Rabbits, Raccoons, and Rats.

Regular daily observations of cottontail rabbits, raccoons, and cotton rats have been made on sprayed and unsprayed islands and dykes since May 13. Observations were recorded on mimeographed forms and have been summarized at weekly intervals. It is hoped that these observations, over a period of time, may be of value in determining whether or not the routine application of DDT is affecting certain mammal populations.

On the sprayed area of dykes and islands, approximately 46.18 acres, 242 observations were made on rabbits, 15 on raccoons, and 40 on cotton rats, from May 13 to June 28. On the unsprayed area, approximately 30.82 acres, 231 observations were made on rabbits, 31 on raccoons, and 33 on cotton rats, over the same period.

The number of observations made by weeks is summarized in table 11.

The variation by weeks in the numbers of observations of animals in table 11 can be accounted for in part by the growth of rabbit litters to the point where the young are able to get about and also to a certain extent by the weather. Observations were made during all types of weather and at all hours of the day. There are no significant differences in the numbers of observations on sprayed and unsprayed dykes that cannot be accounted for.

Table 11. Number of observations made on rabbits, raccoons, and cotton rats.

Date	Sprayed Areas			Unsprayed Areas		
	Rabbit	Raccoon	Rat	Rabbit	Raccoon	Rat
May 13-17	18	4	5	18	1	1
May 20-24	45	0	4	22	7	5
May 27-31	19	2	8	34	4	4
June 3-7	52	2	9	33	5	4
June 10-13	16	2	1	22	1	2
June 17-21	36	3	1	37	6	11
June 24-28	36	2	10	43	0	7
May 13 - June 28	222	15	38	209	24	34

To date no mammals have been found showing effects of DDT poisoning.

Reptiles.

Turtles of four species have been captured by hand on the dykes and islands and marked according to the system devised by F. R. Cagle (Copeia, 3:170 - 173, 1939), which involves filing notches in the marginal plates. Up to June 30, forty-four sliders (Pseudemys scripta scripta) were marked, 19 in sprayed areas and 25 in unsprayed areas. Of the eleven mud turtles (Kinosternum subrubrum subrubrum) marked, seven were from sprayed areas; of the four musk turtles (Sternotheros odoratus) marked, three were from sprayed areas. One of the two snapping turtles (Chelydra serpentina) came from a sprayed area.

The only turtles that have been retaken since the original markings were four Pseudemys scripta. One of these was killed by a raccoon. Attacks by raccoons, in fact, have been the only cause of mortality that has come to our attention. A raccoon kills a turtle by chewing its head or breaking its neck, after which it eviscerates the turtle by pulling the internal organs out through the posterior end of the body.

Sixteen snakes - 14 king snakes (*Lampropeltis getulus*), one black snake (*Coluber constrictor*), and one ribbon snake (*Thamnophis sauritus*) were collected by hand and marked according to the method of Blanchard and Finster (Ecology, 14: 334-347, 1933) which involves removal of caudal scutes by clipping with scissors. Ten of the snakes were taken in sprayed areas and six in unsprayed areas. None has been taken a second time.

EQUIPMENT DEVELOPMENT BRANCH

H. Stierli, J.D. Parkhurst

Household Sprayer

To improve on the one quart size Sure Shot sprayer which had been adapted for residual household application, a lighter and slightly smaller model was made of aluminum. This sprayer was constructed of 52S 1/2H .051 gage aluminum sheet. An all welded type of construction was used, replacing the rivet and solder type assembly of the Sure Shot sprayer. An aluminum hand pump and fittings at the filler inlet also help to reduce weight. The brass valve and cutoff was taken directly from a Sure-Shot sprayer although this may also be made of aluminum if desired.

The dry weight of the aluminum sprayer is only 1 lb. $5\frac{1}{2}$ oz. as compared to 3 lb. 5 oz. of the modified Sure Shot sprayer. One quart of liquid in the sprayer requires 150 strokes of the hand pump for complete dispersal at a pressure of approximately 30-40 psi. and covers an area of 460 sq. ft. when a 6501 Spraying Systems nozzle is used.

The reduced weight of the aluminum sprayer is highly desirable as it substantially lessens tiring of the hand while spraying. Use of the all welded type of aluminum construction permitted a reduction in weight without sacrifice of bonding strength. It is believed that the welded type of construction would also lend itself to production methods so that the sprayer could be manufactured at a reasonable cost.

After testing this sprayer for household spraying, changes in the shape of the handle, the shut off valve, check valve on the hand pump, and hand pump handle seem desirable. Accordingly another model of this type sprayer will be constructed with these changes incorporated in the design.

Portable Power Sprayer.

A sprayer was constructed for application of residual sprays in large buildings, hotels, auditoriums, hospitals, and other structures where the hand sprayer is inadequate. Essentially, the sprayer consists of a small capacity gear pump driven by a direct connected small electric motor, a five gallon aluminum tank, a pressure regulator and bypass, a hose and a spray gun all mounted on an aluminum chassis with rubber tire wheels. In areas where electricity is not available, the electric motor may be replaced by a small air-cooled gasoline engine.

The triangular aluminum chassis is mounted on three rubber tired wheels of five inch diameter and it has overall dimensions of 21 inches wide, 27 inches long. The sprayer stands approximately 20 inches high. A 30 inch handle attached to the movable front wheel permits the sprayer to be pulled and guided in whatever direction desired. The pump outlet is connected with an adjustable spring pressure regulator and bypass. The regulator may be set for any pressure ranging from 30 to 100 psi. The bypass allows the return of unused spray solution to the spray tank, permitting the pump to run continuously even though the spray gun valve is closed. This circulation provides agitation of the spray solution in the tank. Two racks mounted on the chassis will provide fixtures for placement of the spray hose and electric cord when the sprayer is either in transit, or use.

The sprayer is compact, portable, and weighs approximately 50 pounds. One man can easily transport it in a pickup truck. Furthermore, it is small enough to be stored in a closet or storeroom when not in use.

Wheelbarrow Type Sprayer - (Plate 1)

A sprayer was developed for the use of the Texas Dysentery-Fly Control Project to supplement existing hand spraying equipment. It is primarily an outside sprayer, although certain indoor uses may also be found.

Requirements and design considerations were as follows: The sprayer should be of the wheelbarrow type, light, strong, compact, easily portable, of approximately ten gallons liquid capacity, and designed for use with an air compressor mounted on a light pickup truck.

In order that the frame of the sprayer be both strong and light, it was made from 1 inch diameter chrome molybdenum steel tubing. Utilizing a pneumatic wheel $15\frac{1}{2}$ inches in diameter and underslung type of framework, a design was obtained wherein, the center of gravity was low enough to prevent overturning and the sprayer was compact enough to be easily carried in a light truck. Since the sprayer was to be of the compressed air type, two separate tanks, one for spray solution and one for compressed air, were mounted, one behind the other. The tanks were surplus U. S. Army oxygen containers for airplanes, constructed of 1/16 inch stainless steel, to form a cylinder 12 inches in diameter with dome shaped ends, overall length of 24 inches, and capacity of 2100 cubic inches.

Although the original design pressure for these tanks was 400 psi., after installation of adapting fittings in the tanks they were retested for 100 psi. It is not recommended that pressures above 100 psi. be used in the sprayer.

The tank nearest to the wheel, called the spray tank, was fitted with a filler neck and cap, and a pickup pipe with exterior hose fitting, both taken from a Lofstrand hand sprayer, thus enabling replacement gaskets and other parts to be easily obtainable. The other tank, called the air tank, was fitted with a standard Schrader air valve. By connecting the two tanks across the top with 1/4 inch pipe, and installing a cutoff valve, a bleeder valve, and a pressure gage in the system, air pressure could be maintained in the air tank while the spray tank was being filled.

A clip for holding the spray wand and a hose rack capable of holding up to 50 ft. of 3/8 inch diameter spray hose were mounted on the frame work of the wheelbarrow.

Physical data obtained for the sprayer showed that the spray tank held nine gallons of solution. A charge of 85 psi. air pressure in the air tank would spray out the nine gallons of solution with a pressure drop to 40 psi. A No. 8004 Spraying Systems nozzle was employed. The sprayer weighed 74 pounds dry, 149 pounds loaded, and required a lift of 55 pounds at the handles when filled. The overall length was 62 inches and overall width 24 inches.

Portable Power Sprayer.

A sprayer was constructed for application of residual sprays in large buildings, hotels, auditoriums, hospitals, and other structures where the hand sprayer is inadequate. Essentially, the sprayer consists of a small capacity gear pump driven by a direct connected small electric motor, a five gallon aluminum tank, a pressure regulator and bypass, a hose and a spray gun all mounted on an aluminum chassis with rubber tire wheels. In areas where electricity is not available, the electric motor may be replaced by a small air-cooled gasoline engine.

The triangular aluminum chassis is mounted on three rubber tired wheels of five inch diameter and it has overall dimensions of 21 inches wide, 27 inches long. The sprayer stands approximately 20 inches high. A 30 inch handle attached to the movable front wheel permits the sprayer to be pulled and guided in whatever direction desired. The pump outlet is connected with an adjustable spring pressure regulator and bypass. The regulator may be set for any pressure ranging from 30 to 100 psi. The bypass allows the return of unused spray solution to the spray tank, permitting the pump to run continuously even though the spray gun valve is closed. This circulation provides agitation of the spray solution in the tank. Two racks mounted on the chassis will provide fixtures for placement of the spray hose and electric cord when the sprayer is either in transit, or use.

The sprayer is compact, portable, and weighs approximately 50 pounds. One man can easily transport it in a pickup truck. Furthermore, it is small enough to be stored in a closet or storeroom when not in use.

Wheelbarrow Type Sprayer - (Plate 1)

A sprayer was developed for the use of the Texas Dysentery-Fly Control Project to supplement existing hand spraying equipment. It is primarily an outside sprayer, although certain indoor uses may also be found.

Requirements and design considerations were as follows: The sprayer should be of the wheelbarrow type, light, strong, compact, easily portable, of approximately ten gallons liquid capacity, and designed for use with an air compressor mounted on a light pickup truck.

In order that the frame of the sprayer be both strong and light, it was made from 1 inch diameter chrome molybdenum steel tubing. Utilizing a pneumatic wheel 15½ inches in diameter and underslung type of framework, a design was obtained wherein, the center of gravity was low enough to prevent overturning and the sprayer was compact enough to be easily carried in a light truck. Since the sprayer was to be of the compressed air type, two separate tanks, one for spray solution and one for compressed air, were mounted, one behind the other. The tanks were surplus U. S. Army oxygen containers for airplanes, constructed of 1/16 inch stainless steel, to form a cylinder 12 inches in diameter with dome shaped ends, overall length of 24 inches, and capacity of 2100 cubic inches.

Although the original design pressure for these tanks was 400 psi., after installation of adapting fittings in the tanks they were retested for 100 psi. It is not recommended that pressures above 100 psi. be used in the sprayer.

The tank nearest to the wheel, called the spray tank, was fitted with a filler neck and cap, and a pickup pipe with exterior hose fitting, both taken from a Lofstrand hand sprayer, thus enabling replacement gaskets and other parts to be easily obtainable. The other tank, called the air tank, was fitted with a standard Schrader air valve. By connecting the two tanks across the top with 1/4 inch pipe, and installing a cutoff valve, a bleeder valve, and a pressure gage in the system, air pressure could be maintained in the air tank while the spray tank was being filled.

Plate 1. Wheelbarrow type compressed air sprayer of nine gallons liquid capacity.

THE OPERATION AND EVALUATION OF DDT DISPERSAL FROM THE STEARMAN TYPE PT-17 AIRPLANE

H. Stierli, W.R. Schmitz

Preliminary Tests:

During this period considerable time was spent in evaluating the performance of the Stearman Type 220 H.P. PT-17 airplane. Numerous tests were made with both the spray and the thermal aerosol type dispersal of 20 percent (by weight) DDT in Velsicol NR-70.

A portable weather tower, 6 1/2 feet tall, was constructed to facilitate accurate measurement of essential meteorological variables during tests. Air temperatures were observed at 6 feet and 1 foot above the ground level using paired mercury thermometers shielded from the direct radiation of the sun. Average wind velocities were secured over one minute periods with a 4 inch diameter pinwheel anemometer at the 6 foot level. A sensitive vane with removable pivot was mounted on the top of the tower for observation of wind direction. The difference in temperature of the air at 6 feet and 1 foot above ground is an indication of vertical thermal air movement. Downward air movement occurs during a condition known as "inversion" (T_6-T_1 a plus value). Thermal upward movement of air normally occurs during a condition of "lapse" (T_6-T_1 a negative value). It is generally accepted that best deposition of small spray particles is obtained during conditions of "inversion".

Carbon-coated slides were used to sample the deposited droplets, and the mass median diameter and area dose were determined by counting. For each test, two lines of stations, about 100 feet apart, were set-up; the stations in each line were 25 feet apart. In summarizing the data, the values for the two corresponding stations were averaged and recorded as one figure.

Cross-wind flights of the airplane were made using three new nozzle arrangements. Each set of nozzles was tested twice, and the mass median diameter and area dose at various distances from the line of flight were determined. The results of these tests are summarized in tables 12a and 12b. The test data indicate a non-uniform distribution of spray regardless of the nozzle arrangement employed. Furthermore, it appears that any of the alternate arrangements could be used satisfactorily. It was noted that with a light to medium breeze, the first drops were deposited on the ground about 25 to 75 feet from the line of flight of the airplane, depending upon the elevation of flight and wind velocity. The original and present arrangement of nozzles (shown in test 1) being used consists of five Spraying Systems Co. No. 1/4LN12 Nozzles.

Following the recommendations of TVA, the discharge section of the exhaust venturi was reduced from 22 1/2 inches in length to 10 inches. The effects of shortening the venturi were tested by several cross-wind flights; but the data showed no significant change in particle size, recovery, or distribution. The results of the tests are summarized in tables 13a and 13b. It was noted that the thermal aerosol with a light to medium breeze, deposited the first drops about 40 to 140 feet from the line of flight of the airplane, depending upon the elevation of flight and the wind velocity.

Multiple swath tests, with both the spray and thermal aerosol, were also made. Two rows of slides were placed perpendicular to the lines of flight and extended for 500 feet. The airplane made four flights, each at 100 feet intervals, as indicated by the diagram in table 14a. In general, with spray applications better recovery of DDT was obtained, but the distribution was less uniform. The thermal aerosol gave better distribution than the spray but the recovery was poorer. In making multiple swaths with the airplane,

Table 12a. Operational and meteorological data for comparative spray nozzle tests on Stearman type PT-17 airplane, April 2, 1946.

Test Number	Time	Solution Pressure (psi)	Rate of Discharge (gpm)	Altitude of Flight (ft)	Meteorological Conditions				
					T_6 (°F)	T_1 (°F)	$T_6 - T_1$	V_6 (mph)	Wind Direction
Five Atomizing Nozzles, Spraying Systems Co. No. 1/4 LN12 (Four on lower wing, one on tail)									
1	6:47 AM	49	1.2	20	56.1	55.8	0.3	2.4	S
Three Atomizing Nozzles, Spraying Systems Co. No. 1/4 LN26 (Two on wing tips, one on tail)									
3	7:08	51	1.2	25	58.4	58.4	0.0	3.3	SW
4	7:14	50	1.2	35	59.2	59.2	0.0	5.4	SW
Two Atomizing Nozzles, Spraying Systems Co. No. 1/4 LN26 (On wing tips)									
5	7:24	50	0.9	30	60.0	60.0	0.0	5.2	SW
6	7:30	50	0.9	30	60.6	60.8	-0.2	7.2	SSW
Two Flat Atomizing Nozzles, Spraying Systems Co. No. 1/4 T8006 (On wing tips)									
7	7:40	50	1.4	30	61.6	62.0	-0.4	6.8	SSW
8	7:47	47	1.4	30	62.2	62.8	-0.6	7.5	SSW

Notations:
- T_1-- Temperature 1 foot above ground level
- T_6-- Temperature 6 feet above ground level
- V_6-- Wind velocity 6 feet above ground level

Remarks:
Official Sunrise was 6:13 A.M. However, ground fog kept the airplane grounded until 6:30 A.M. All flights were from East to West. Airplane speed was approximately 90 miles per hour.

Table 12b. Recovered dosage and mass median diameter obtained in spray application of 20 percent DDT in Volsicol NR70 by PT-17 airplane, April 2, 1946

		Upwind		Downwind								
Test No.	Distance foot	25	0	25	50	75	100	125	150	175	200	225
1	Area Dose lb. DDT/acre	0	0	0.543	0.057	0.030	0.016	0.011	0.007	0	0	0
	MMD Microns	0	0	620	99	255	50	70	50	0	0	0
3	Area Dose lb. DDT/acre	0	0	0.100	0.093	0.086	0.055	0.181	0.015	0.012	.010	0
	MMD Microns	0	0	200	150	259	170	180	115	67	65	0
4	Area Dose lb. DDT/acre	0	0	0	0.193	0.260	0.231	0.013	0.004	0.091	0.007	0.010
	MMD Microns	0	0	0	345	300	250	100	33	220	40	44
5	Area Dose lb. DDT/acre	0	0	0	0.079	0.502	0.179	0.088	0.042	0.043	0.004	0.004
	MMD Microns	0	0	0	95	425	260	240	170	110	50	48
6	Area Dose lb. DDT/acre	0	0	0	0.011	0.034	0.013	0.085	0.096	0.079	0.188	0.028
	MMD Microns	0	0	0	200	170	210	210	195	225	150	160
7	Area Dose lb. DDT/acre	0	0	0.009	0.317	0.062	0.006	0.132	0.025	0.006	0.036	0.010
	MMD Microns	0	0	125	375	170	100	142	135	33	115	150
8	Area Dose lb. DDT/acre	0	0	0	0	0.250	0.013	0.048	0.031	0.126	0.039	0.012
	MMD Microns	0	0	0	0	390	100	205	63	105	140	75

Notes: 1. Each figure is an average of two determinations.
2. Each flight was cross-wind.
3. All area dose calculations are based on the counting method of analysis.

Table 13a. Operational and meteorological data for comparative aerosol tests on the original and shortened venturi discharge section of the Stearman type PT-17 airplane, April 3, 1946.

Test Number	Time	Solution Pressure (psi)	Rate of Discharge (gpm)	Altitude of Flight (ft)	Meteorological Conditions				
					T_6 (°F)	T_1 (°F)	$T_6 - T_1$	V_6 (mph)	Wind Direction
	Original Length of Discharge Section 22 1/2" from venturi throat to end								
1	6:18 AM	40	1.1	30	58.4	57.6	0.8	4.3	W
2	6:26	40	1.1	30	58.6	58.4	0.2	4.0	W
			Discharge Section Cut to 16" Length						
3	6:40	40	1.1	35	60.0	59.8	0.2	3.2	W
4	6:46	40	1.1	20	60.4	60.2	0.2	3.3	W
			Discharge Section Cut to 10" Length						
5	7:02	40	1.1	20	60.8	60.8	0.0	4.6	W
6	7:09	40	1.1	20	61.0	61.0	0.0	4.6	WSW
7	7:18	40	1.1	20	62.0	62.0	0.0	5.8	WSW

Remarks: Official sunrise was 6:11 A.M.
Sky was clear and sunny.
All flights were from South to North.

Table 13b. Recovered dosage and mass median diameter obtained in thermal aerosol application of 20 percent DDT in Velsicol NR70 by PT-17 airplane, April 3, 1946.

		Upwind	Downwind									
Test Number	Distance Feet	10	40	65	90	115	140	165	190	215	240	290
1 Long Venturi	Area Dose lb DDT/acre	0	0	0	.003	.001	.002	.001	.006	.003	.003	.004
	MMD Microns	0	0	0	40	50	35	35	51	41	40	51
2 Long Venturi	Area Dose lb. DDT/acre	0	0	0	0	.004	.004	.003	.004	.002	.002	.003
	MMD Microns	0	0	0	0	52	53	48	50	45	42	53
3 Inter. Venturi	Area Dose lb.DDT/acre	0	0	0	0	0	.006	.004	.003	.006	.004	.001
	MMD Microns	0	0	0	0	0	57	51	42	62	43	32
4 Inter. Venturi	Area Dose lb.DDT/acre	0	0	.016	.005	.008	.002	.004	.008	.014	.013	.029
	MMD Microns	0	0	73	51	52	37	49	56	58	65	86
5 Short Venturi	Area Dose lb.DDT/acre	0	0	.011	.020	.014	.005	.003	.001	.008	.003	.003
	MMD Microns	0	0	50	58	63	54	47	30	72	33	43
6 Short Venturi	Area Dose lb.DDT/acre	0	.001	.009	.004	.008	.003	.003	.003	.001	.003	.001
	MMD Microns	0	25	61	43	54	40	40	39	36	47	34
7 Short Venturi	Area Dose lb.DDT/acre	0	0	.007	.002	.004	.005	.003	.002	.001	.001	.004
	MMD Microns	0	0	100	45	46	50	47	35	25	31	53

NOTES: 1. Each figure is an average of two figures.
2. Each flight was cross-wind.
3. All area dose calculations are based on the counting method of analysis.

much of the dispersed DDT overlapped previous swaths made by the airplane. With the thermal aerosol it was observed that the white cloud continued to move along close to the ground for several hundred feed beyond the slides. The slides which were exposed to all of the swaths of the airplane tended to have more DDT deposited on them than the slides which were only exposed to one swath of the airplane. In treating large areas, the overlapping of swaths gives better distribution and also better recovery of the DDT dispersed. Multiple swaths of the spray generally show a peak deposition of DDT on slides 25 feet downwind from the line of flight and a marked reduction in deposition of DDT on slides 75 or 100 feet from the line of flight. The results of multiple cross-wind swath tests with both spray and thermal aerosol are summarized in tables 14a and 14b. It was noted that apparently satisfactory recovery of both sprays and aerosols was obtained during application with surface winds as much as six miles per hour. The swaths were displaced 25 to 50 feet, which does not appear excessive for routine treatments of moderate to large size plots. However, it must be realized that these tests were performed during marked conditions of "inversion" and with the airplane at very low altitude.

Treatment of the Savannah Wildlife Refuge:

Weekly treatment of four Savannah Wildlife Refuge plots for studies on the effects of DDT on aquatic and terrestrial forms of life was begun on May 1, 1946. In order to treat the pools with spray and aerosol under similar conditions two airplanes are utilized. One airplane applies spray to pool 6 (270 acres)* and pool 2 (185 acres) while the other disperses the exhaust aerosol to pool 3 A (120 acres) and pool 3 (240 acres).

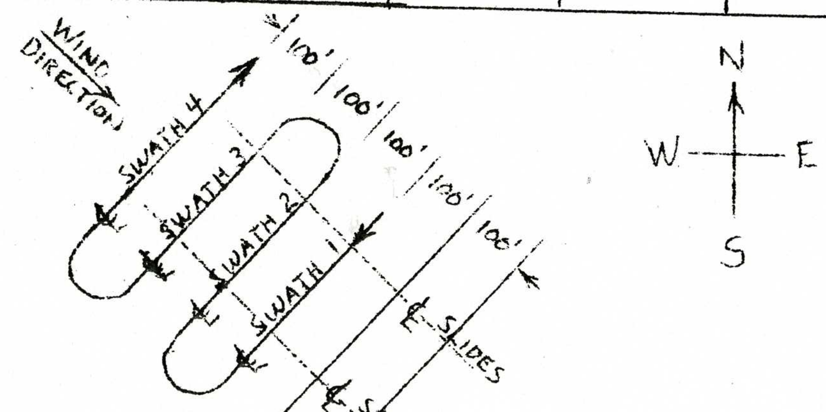
* Treatment area includes the pool area plus a strip approximately 100 feet wide around the pool.

Table 14a. Operational and meteorological data for multiple swath applications of sprays and exhaust aerosols with the Stearman type PT-17 airplane, April 5, 1946.

Test Number	Time	Solution Pressure (psi)	Rate of Discharge (gpm)	Altitude of Flight (ft)	Meteorological Data				
					T_6 (°F)	T_1 (°F)	$T_6 - T_1$	V_6 (mph)	Wind Direction
1 Aerosol	6:14 AM	40	1.2	25	57.0	54.8	2.2	6.2	NW
2 Spray	6:27	40	1.0	20	56.6	54.8	1.8	5.5	NW
3 Aerosol	6:38	41	1.1	20	56.6	54.2	2.4	3.8	NW
4 Spray	6:49	41	0.9	20	56.6	54.0	2.6	4.2	NW

REMARKS:

Official sunrise was 6:09 A.M.
Sky was clear and sunny during application except for Test No. 3 in which a cloud blocked the sun.



Flight Pattern

Table 14b. Recovered dosage and mass median diameter obtained in multiple swath application of 20 percent DDT in Velsicol NR70 by PT-17 airplane, April 5, 1946.

Distance, feet Lines of flight indicated by arrows	TEST NO. 1 Thermal Aerosol		TEST NO. 2 Spray		TEST NO. 3 Thermal Aerosol		TEST NO. 4 Spray	
	MMD Microns	Area Dose lb.DDT/acre	MMD Microns	Area Dose lb.DDT/acre	MMD Microns	Area Dose lb.DDT/acre	MMD Microns	Area Dose lb.DDT/acre
→ 0	0	0	0	0	0	0	0	0
25	0	0	365	.328	0	0	131	.040
50	28	.005	121	.204	71	.014	208	.040
75	38	.020	152	.097	51	.006	112	.045
→ 100	44	.005	48	.011	46	.006	80	.012
125	57	.016	334	.204	51	.008	325	.305
150	50	.007	193	.052	58	.015	137	.089
175	45	.005	69	.036	59	.015	166	.040
→ 200	43	.006	165	.463	48	.010	164	.161
225	75	.019	253	.049	50	.027	272	.190
250	63	.019	149	.131	46	.020	104	.042
275	47	.008	139	.099	50	.018	119	.062
→ 300	63	.015	142	.101	42	.010	247	.197
325	72	.017	407	.415	76	.050	271	.293
Wind 350	60	.010	400	.311	63	.038	130	.126
Direction 375	42	.004	93	.031	44	.014	104	.041
↓ 400	62	.015	103	.055	47	.014	94	.021
425	53	.006	67	.027	48	.013	46	.014
450	73	.014	63	.016	49	.011	74	.022
475	79	.025	92	.041	63	.023	138	.027
500	63	.015	97	.032	39	.012	95	.018

NOTES: 1. Each figure is an average of two figures.
2. Cross-wind flight at 100 feet intervals.
3. All area dose calculations are based on the counting method of analysis.

The output dosage for both spray and aerosol is 0.1 lb. DDT per acre, employing a solution containing 20 percent (by weight) DDT in Velsicol NR70. Applications are made at an elevation of about 30 feet wherever possible, and roughly 20 feet above trees and other obstructions. Uniformly spaced and parallel swaths of 100 feet width are obtained by using existing landmarks and twenty-five 4 ft. x 4 ft. yellow markers strategically placed for guidance of the pilot. The markers were installed at 500 feet intervals perpendicular to the lines of flight. The pilot can fly over a marker for his first, sixth, eleventh, etc., swath with satisfactory spacing of intermediate swaths. To minimize natural flight hazzards it was necessary to remove several tall dead trees from pools 6 and 3 A.

The treatment time for pools 6 and 2 are about 27 minutes each for a total of approximately 50 to 55 minutes. Pools 3 and 3A are treated as a unit and normally require 45 to 50 minutes for application.

Table 15 presents a typical log of operations for the spray and aerosol treatments at the Savannah Wildlife Refuge.

The evaluation of the amount of DDT actually deposited is determined by chemical analysis of the DDT deposited on 3 inch x 12 inch glass panels. On each of the ponds, twelve stakes were placed in a "horseshoe type" arrangement with a small platform nailed on top of each stake to hold the glass panel. Two dikes along the ponds were also selected for sampling stations, and six stations were placed on each dike.

Initially it was desired to use carbon-coated slides to sample the deposited DDT, with only an occasional check by actual chemical analysis of DDT. Because of the time required to place slides or glass panels on each of the four ponds and along two dikes, it was necessary to place the slides or panels on the stations the day before the actual airplane treatment, which occurs at dawn. Because of heavy dew or rain, too many of the carbon-coated slides were

Table 15. Log of airplane spray and aerosol operations at the Savannah Wildlife Refuge, June 18, 1946

Time	T_6	T_1	$T_6 - T_1$	V_6	Wind Direction	Remarks
5:30	71.4	70.8	0.6	2.0	WSW	5:18 A.M. Official sunrise Sun emerging from clouds.
5:40	71.4	71.0	0.4	2.2	SW	5:30 Sky mostly clear; but some haziness and ground fog. Taylor start aerosol Pools 3 & 3A
5:50	71.8	71.2	0.6	2.5	SW	5:34 Johnson start spray Pool 6
6:00	72.0	72.0	0.0	2.4	SW	6:01 Johnson finish Pool 6 (27 min.)
6:10	72.8	72.8	0.0	2.8	WSW	6:02 Johnson start Pool 2
6:20	73.2	73.8	-0.6	1.8	SW	6:20 Taylor finish Pools 3 & 3A (50 min)
6:30	73.8	74.0	-0.2	3.2	SW	6:29 Johnson finish Pool 2 (27 min.)
Maximum	73.8	74.0	0.6	3.2		
Average	72.3	72.2	0.1	2.4	SW	
Minimum	71.4	70.8	-0.6	1.8		

DOSAGES:
Spray : 25.1 gals. for 455 acres; 0.10 lb.DDT/acre
Aerosol: 24.0 gals. for 360 acres; 0.12 lb.DDT/acre

ruined, and this method of analysis had to be abandoned in favor of quantitative chemical analysis of DDT.

Special boxes similar to a microscope slide box were constructed to hold the 3 inch by 12 inch glass panels. Acetone was used to wash the DDT off the panels into beakers. The washings were then placed in test tubes and the entire amount used for analysis. A colorimetric procedure based upon nitration of DDT and addition of sodium methylate to develop the color was used for DDT analysis.

The ponds are fairly open and free from obstructions except along the dikes which are grown up in varying degrees with vegetation, brush, trees, etc. The dikes along pool 6 is particularly dense. Considerable "saw grass", lotus and lily pads are in each pond, but these do not interfere with the flight of the airplane, or sampling of the deposited DDT.

Table 16a presents the maximum, minimum, and average meteorological readings obtained during applications. The recovered dosage for each pond and dike is reported in tables 16b and 16c as the average of the twelve stations on the ponds and the average of six stations on the dikes. The standard error of the mean for each pond and the dike is also given. With the spray type dispersal of DDT, the recovered dosages have ranged from a low of 37 percent to a high of 86 percent recovery on the ponds and from 13 percent to 82 percent recovery on the dike. With the thermal aerosol type dispersal of DDT, the recovered dosages have ranged from a low of 5 percent to a high of 25 percent recovery on the ponds and from 1 percent to 18 percent recovery on the dike. Although the spray gives a much higher recovery of DDT, the distribution is somewhat spotty. Occasionally, stations which are no more than 30 feet apart will vary in dosage by more than a hundred fold. The thermal aerosol gave a more uniform distribution, but the recovered dosage was also much lower. Roughly four times more DDT was recovered from the spray than

Table 16a. Summary of meteorological data and dosages for airplane spray and aerosol applications at the Savannah Wildlife Refuge statring May 1, through June 26, 1946.

Date		Meteorological Conditions T_6	$T_6 - T_1$	V_6 (mph)	Wind Direction	Output Dosage lbs. DDT/acre Spray	Aerosol	Remarks
5/1	Max.	69.0	-1.6	4.3				
	Av.	68.5	-1.7	3.1	NE	0.10		
	Min.	68.0	-1.8	1.9				
5/9	Max.	56.6	1.2	2.9				Solution pressure was too low for the desired output.
	Av.	56.1	0.1	2.2	WSW	0.07		
	Min.	55.8	-0.8	1.3				
5/21	Max.	67.0	0.2	5.2				
	Av.	66.0	-0.2	3.7	W	0.10	0.10	
	Min.	65.8	-1.0	2.1				
5/28	Max.	63.4	1.0	6.3				Aerosol nozzles were partially clogged at end of treatment.
	Av.	60.6	0.2	4.7	W	0.10	0.09	
	Min.	59.0	-0.6	2.6				
6/4	Max.	67.2	0.0	4.6				Aerosol nozzles were partially clogged.
	Av.	67.0	-0.1	4.1	N	0.10	0.05	
	Min.	66.8	-0.6	3.0				
6/11	Max.	76.0	2.0					New aerosol nozzle tips installed.
	Av.	73.4	0.9	1.0	ENE	0.10	0.13	
	Min.	71.0	-0.4					
6/18	Max.	73.8	0.6	3.2				
	Av.	72.3	0.1	2.4	SW	0.10	0.12	
	Min.	71.4	-0.6	1.8				
6/25	Max.	68.5	0.7	3.5				
	Av.	67.5	0.4	2.8	NNW	0.10	0.12	
	Min.	66.2	-0.3	1.6				

NOTE: Rain and other unfavorable metorological conditions prevented treatment during the week beginning May 13th.

Table 16b. Recovered dosage obtained in thermal aerosol applications of 20 percent DDT in Velsicol NR70 by PT-17 airplane at the Savannah Wildlife Refuge.

DATE	Dosage Applied lb. DDT/acre	POOL # 3A			POOL # 3			DIKE ALONG POOL #3 - #3A		
		Dosage Recovered lb.(1) DDT/acre	Standard Error lb. DDT/acre	Percent Recovery	Dosage Recovered lb.(1) DDT/acre	Standard Error lb. DDT/acre	Percent Recovery	Dosage Recovered lb.(2) DDT/acre	Standard Error lb. DDT/acre	Percent Recovery
May 21,1946	0.10	0.005	± 0.001	5%	0.012	± 0.005	12%	0.006	± 0.002	6%
May 28,1946	0.09	0.008	± 0.002	9%	0.005	± 0.001	6%	0.0012	± 0.0002	1.3%
June 4,1946	0.05	0.003	± 0.001	6%	0.008	± 0.001	16%			
June 11,1946	0.13	0.033	± 0.007	25%	0.031	± 0.004	24%	0.023	± 0.008	18%
June 18,1946	0.12	0.014	± 0.005	12%	0.008	± 0.002	7%	0.010	± 0.002	8%
June 25,1946	0.12	0.011	± 0.003	9%	0.013	± 0.002	11%	0.016	± 0.003	13%

NOTES: 1. Average of 12 stations.
2. Average of 6 stations.
3. All dosage calculations are based on quantitative chemical analysis of DDT.

Table 16c. Recovered dosage obtained in spray applications of 20 percent DDT in Velsicol NR70 by PT-17 airplane at the Savannah Wildlife Refuge.

DATE	Dosage Applied lb. DDT/acre	POOL # 6			POOL # 2			DIKE ALONG POOL # 6		
		Dosage Recovered lb.(1) DDT/acre	Standard Error lb. DDT/acre	Percent Recovery	Dosage Recovered lb.(1) DDT/acre	Standard Error lb. DDT/acre	Percent Recovery	Dosage Recovered lb.(2) DDT/acre	Standard Error lb. DDT/acre	Percent Recovery
May 1, 1946	0.10							0.060	± 0.017	60%
May 9, 1946	0.07	0.032	± 0.005	46%						
May 21,1946	0.10	0.041	± 0.010	41%	0.050	± 0.007	50%	0.028	± 0.012	28%
May 28,1946	0.10	0.046	± 0.010	46%	0.086	± 0.021	86%	0.013	± 0.004	13%
June 4,1946	0.10	0.063	± 0.019	63%	0.038	± 0.010	38%	0.050	± 0.029	50%
June 11, 1946	0.10	0.044	± 0.009	44%	0.069	± 0.012	69%	0.038	± 0.008	38%
June 18,1946	0.10	0.037	± 0.003	37%	0.047	± 0.016	47%	0.014	± 0.008	14%
June 25,1946	0.10	0.038	± 0.003	38%	0.083	± 0.018	83%	0.082	± 0.049	82%

NOTES: 1. Average of 12 stations.
2. Average of 6 stations.
3. All dosage calculations are based on quantitative chemical analysis of DDT.

from the thermal aerosol. It appears significant that the best recovery of thermal aerosol occurred on June 6, when the average wind velocity did not exceed 1 m.p.h. during application and the average temperature differential ($T_6-T_1 = 0.9^o$) was greatest.

Treatment of a Forest Plot:

Four satisfactory spray applications of 30 percent DDT in Velsicol NR70 were made on a 57 acre forest plot at Camp Stewart, Georgia. Three Spraying Systems Co. fan pattern nozzles No. 1/4T8010 with pressure of 70 psi. were employed. The spray was dispersed at the rate of 3.25 gallons per minute using swaths of 50 feet to obtain an output dosage of 1.0 lb. DDT per acre per application. Uniform swaths were secured by flying in one direction and parallel to a highway bordering the plot when dispersing the spray. A 4 ft. X 6 ft. yellow plywood panel was moved perpendicular to the line of flight as a guide to the pilot for spacing his swaths. Although the spray drops were fairly large, the distribution over the area was good and a substantial penetration through the trees and underbrush was observed. The insect population in the area was practically eliminated shortly after treatment. However, reinfestation from the untreated surroundings occurred during the weekly interval of spraying.

CHEMICAL INVESTIGATIONS BRANCH

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Chemical Deterioration of DDT in Residual Spraying

From information available, it was concluded that DDT applied as a residual spray at the rate of 200 milligrams per square foot should remain toxic to insects longer than the present effective period. Since flies, mosquitoes and other insects probably remove relatively little of the DDT applied, a series of tests have been set up to determine what factors are most influential in the deterioration of DDT.

Three types of surfaces, wood, paper, and glass, were selected to be treated with both 5 percent DDT in kerosene, and the standard U.S.P.H.S. 5 percent DDT in xylene emulsion, hereafter referred to as emulsion. The wood panels were made from poplar wood, and the paper panels were wood panels covered tightly, but not pasted, with wallpaper. All of the panels were 3" x 12" and were sprayed by a micro-sprayer. This sprayer set-up consisted of a Spraying Systems Co. siphon type atomizing nozzle which mixed the compressed air and liquid externally. The sprayer was set with nozzle pointing downward, and with a 5 milliliter pipette bent at right angles, attached to the liquid feed side. A small air pressure of 30 to 40 millimeters of mercury was used to minimize bouncing of drops from the panels. The nozzle was placed about 3/4 inch above the surface of the panel. The panels were moved by hand on a tract which kept them always under the nozzle. With this apparatus, it was possible to spray exactly one milliliter of solution or 50 milligrams of DDT on each panel.

The DDT on the wood panels was removed by scraping off the top 1/8 inch of the surface and extracting for four hours with benzene in a Soxhlet extractor. The DDT on the paper panels was removed by cutting the paper around the edge of the panel and extracting with benzene in a Soxhlet extractor for two hours. The glass panels were simply washed with benzene to remove the DDT. A colorimetric procedure based upon nitration of DDT and addition of sodium methylate to develop the color was used for DDT analysis.

Triplicate panels with both 5 percent DDT in kerosene and 5 percent DDT emulsion on glass, paper, and wood surface were prepared and an initial analysis made on each to determine the recovery from each type panel. The results are summarized and recorded as test 1 in table 17. In all cases better than 90 percent of the DDT was recovered.

As a control for the other tests (test 2, table 17) duplicate panels with 5 percent DDT in kerosene and 5 percent DDT emulsion on glass, paper, and wood surface were prepared and placed in the dark for a period of one month at room temperature. The DDT on these panels were thus subject chiefly to varying room temperature and aging. It is interesting to note that loss of DDT from kerosene-glass panels was quite appreciable, with 63 percent recovery obtained. On the other type panels only a slight difference was found from the initial analysis.

The effect of high temperature on DDT was determined (test 3, table 17) by placing duplicate or triplicate panels with 5 percent DDT in kerosene and 5 percent DDT emulsion on glass, paper, and wood surface in an oven at a constant temperature of 140 degrees Fahrenheit for one week. Because of the capacity of the oven, it was impossible to run more than four panels at one time. On the kerosene-glass panels only about 9 percent of the DDT was recovered as compared to 100 percent for initial analysis and 63 percent recovery for kerosene-glass panels in the control series. The paper-kerosene panels and wood-kerosene panels gave respectively 53 percent and 49 percent recovery as compared to 85 percent and 86 percent recovery in the control

Table 17. Chemical deterioration of DDT in residual spraying.

Description of Test	5% DDT Solution of	Surface	Amount of panel analyzed	Dosage DDT Applied	Dosage DDT Recovered (1)	Percent DDT Recovery
1. Initial analysis to establish recovery rate for different type panels. Run in triplicate.	Kerosene	Glass	All	50 mg	50.1 mg	100 %
	Kerosene	Paper	Top Layer 1/8"	50 mg	45.4 mg	91 %
	Kerosene	Wood		50 mg	46.2 mg	92 %
	Emulsion	Glass	All	50 mg	50.5 mg	101 %
	Emulsion	Paper	Top Layer 1/8"	50 mg	48.8 mg	97 %
	Emulsion	Wood		50 mg	47.8 mg	95 %
2. Control panels. Analyzed one month after being sprayed. Run in duplicate. Kept in dark place at room temperature.	Kerosene	Glass	All	50 mg	31.7 mg	63 %
	Kerosene	Paper	Top Layer 1/8"	50 mg	42.7 mg	85 %
	Kerosene	Wood		50 mg	42.8 mg	86 %
	Emulsion	Glass	All	50 mg	48.5 mg	97 %
	Emulsion	Paper	Top Layer 1/8"	50 mg	48.5 mg	97 %
	Emulsion	Wood		50 mg	45.2 mg	90 %
3. Hold at constant temperature of 140°F for one week. Glass and wood run in triplicate; paper run in duplicate.	Kerosene	Glass	All	50 mg	4.7 mg	9 %
	Kerosene	Paper	Top Layer 1/8"	50 mg	26.7 mg	53 %
	Kerosene	Wood		50 mg	24.6 mg	49 %
	Emulsion	Glass	All	50 mg	32.2 mg	64 %
	Emulsion	Paper	Top Layer 1/8"	50 mg	34.0 mg	68 %
	Emulsion	Wood		50 mg	39.1 mg	78 %
4. Hold at constant temperature of 40°F for one month. Run in duplicate.	Kerosene	Glass	All	50 mg	37.8 mg	76 %
	Emulsion	Glass	All	50 mg	52.5 mg	105 %
5. Exposed to ultra-violet light for 248 hours or 31 eight hour days. Run in duplicate.	Kerosene	Glass	All	50 mg	20.8 mg	41 %
	Emulsion	Glass	All	50 mg	32.4 mg	65 %
6. Flaking tests. Subjected to 42 severe artificial bumps over a period of one month. Run in duplicate. Dosage DDT recovered is actual amount of DDT flaked off of each panel	Kerosene	Glass	Each panel was covered DDT which had actually flaked off was analyzed	50 mg	0.23 mg	0.5%
	Kerosene	Paper		50 mg	0.14 mg	0.3%
	Kerosene	Wood		50 mg	0.10 mg	0.2%
	Emulsion	Glass		50 mg	0.15 mg	0.3%
	Emulsion	Paper		50 mg	0.09 mg	0.2%
	Emulsion	Wood		50 mg	0.09 mg	0.2%
7. Humidity Tests — (A) Exposed to dry air at room temperature for one month in dark place. Run in duplicate.	Kerosene	Glass	All	25 mg	16.5 mg	66 %
	Emulsion	Glass	All	25 mg	25.8 mg	103 %
7. Humidity Tests — (B) Exposed to saturated air at room temperature for one month in dark place. Run in duplicate.	Kerosene	Glass	All	25 mg	18.6 mg	74 %
	Emulsion	Glass	All	25 mg	24.6 mg	98 %

(1) Standard error is $\pm$ 1.64 mg. for those run in triplicate and $\pm$ 2.00 mg. for those run in duplicate.

series, while emulsion-glass, -paper, and-wood panels gave respectively 64 percent, 68 percent, and 78 percent recovery as compared to 97 percent, 97 percent, and 90 percent in the control series. The recovery of DDT applied in kerosene solution is significantly lower than DDT applied in emulsion form.

Duplicate panels with 5 percent DDT in kerosene and 5 percent DDT emulsion on glass surface only were held for one month at a constant temperature of 40 degrees Fahrenheit (test 4, table 17). The kerosene-glass panels gave 76 percent recovery of DDT, while the emulsion glass panels gave 100 percent recovery of DDT. This recovery of DDT is slightly better than for the corresponding control panels, showing no loss of DDT when subjected to cool temperatures.

Duplicate panels with 5 percent DDT in kerosene and 5 percent DDT emulsion on glass surface only were prepared and exposed to ultra-violet light for 248 hours, or 31 eight hour days (test 5, table 17). These panels were placed in a box and thus all other light was shut out except for the ultra-violet light. The light was not operated continuously, but for 8 hour periods. The kerosene-glass panels gave 41 percent recovery of DDT and emulsion-glass panels gave 65 percent recovery. This indicates that ultra-violet light can cause loss of DDT possibly by acting as a catalyst or perhaps by producing enough heat to cause the loss of DDT. There was no circulation of air in the box containing the panels and after long periods of exposure the temperature reached as high as 113 degrees Fahrenheit.

Flaking tests were also conducted to determine how much DDT is lost by flaking (test 6, table 17). Duplicate panels with 5 percent DDT in kerosene and 5 percent DDT emulsion on glass, paper, and wood surfaces were prepared. Each panel was covered with a heavy grade filter paper and sealed along the edge and back with scotch tape. This covering was about ½ inch from the surface of the panels. All panels were mounted in a single line on a large piece of plywood and suspended by hinges at the top. This enabled the plywood to be raised 18 inches from the wall and dropped giving each panel the same degree of vibration. This was done twice a day for 42 times over a period of one month. Any DDT flaked off had to remain within the cover, which after a month's time, was removed and the content analyzed for DDT. In all cases, it was found that less than 0.5 percent of the DDT applied had flaked off. This indicates that very little DDT flakes off during the first month after spraying, and that if flaking occurs to any considerable extent, it must occur after greater aging of DDT crystals.

The effect of humidity was tested by placing duplicate panels with 5 percent DDT in kerosene and 5 percent DDT emulsion on glass surface only, in both dry air and saturated air (test 7, table 17). One large dessicator was filled with calcium chloride in the bottom to provide dry air, and another large dessicator was filled with water in the bottom to provide saturated air. Both dessicators were kept dark inside a box at room temperature. At the end of one month exposure in dry air, the kerosene-glass panels gave 66 percent DDT recovery and the emulsion-glass panels gave 100 percent recovery. This is essentially the same as the recovery obtained from the panels exposed for one month to saturated air, the kerosene-glass panels gave 74 percent DDT recovery and the emulsion-glass panels gave 98 percent recovery. Thus, little effect of humidity on DDT was detected.

In all of the tests made thus far, better recovery of DDT was obtained when using the emulsion form rather than the kerosene solution, the over-all means being 43.69 mg. of DDT per panel for the emulsion and 35.73 mg. for the kerosene solution, the difference far exceeding the one percent level of significance as determined by the t-test. Significantly less DDT was recovered from the glass panels (mean of 35.77 mg.) than from the paper (43.66 mg.) or wood (42.45 mg.) panels, there being no significant difference between the latter two. It is noteworthy that in test 1, the initial analyses from glass were higher than either paper or wood and these figures are actually included in the means given above. Despite this fact the difference exceeds five per-cent level of significance.

This experiment was designed so that each test would run over a period of three months, with analyses being made at the end of one month and three months' time. The results reported are for only the one month period. Also, tests are simultaneously being run to determine the effect of insect activity on the deterioration of DDT.

Water Wettable DDT Powders

Several commercial water wettable DDT powders were tested and a comparison made. (See table 18) The factors considered on each powder were the DDT content, particle size, wettability, stability, flocculation, and the facility with which it passed through a spray nozzle.

The DDT content as stated by the manufacturer was considered to be correct and no chemical analyses were made for DDT in the various powders. The data on particle size was obtained by screening each powder through a 325 mesh or 44 micron screen with tap water. The results on particle size are recorded as percent by weight of the powder less than 44 microns in diameter. An attempt was made to screen each powder in the dry state, but the tremendous number of large aggregates of small particles made it impractical. In water, most of the aggregates were broken up and would then readily pass a 44 micron screen.

Wettability, stability, and flocculation were determined by adding the DDT powder to 100 milliliters of tap water in a 500 milliliter bottle to make

Table 18. Water wettable DDT.

No.	DDT Content	Particle Size (1) %<44 mu	Wettability No. of shakes to wet DDT powder	Stability time, mins.(2) Initial 30 shakes	Settle for 30 mins. and reshake 30 times	Flocculation	Minimum Requirements for spraying (3) Pressure lb/sq.in.	Nozzle Opening	Remarks
1	50%	60%	>100	1 min.(4)	1 min.(4)	Settles to bottom	50 30	80-06 80-10	Foams too much
2	50%	65%	>100	<1 min.(4)	1 min.(4)	Cluster of particles at top and bottom	40 10	80-04 80-06	Not stable.
3	50%	75%	>100	<1 min.(4)	<1 min.(4)	Cluster of particles at top and bottom	10	80-02	Not stable.
4	25%	95%	40	2 min.(4)	3 min.	Settles to bottom	20 10	80-02 80-04	Foams, but not serious. Warrants Field Testing.
5	50%	95%	20	$1\frac{1}{2}$ min.	$1\frac{1}{2}$ min.	Settles to bottom	10	80-02	Easily wet, but poor stability.
6	50%	95%	20	$1\frac{1}{2}$ min.	2 min.	Settles to bottom.	10	80-02	Easily wet, but poor stability.
7	50%	100%	20	2 min.	1 min.	Settles to bottom	20	80-02	Continued or violent shaking coagulates DDT and settles immediately.
8	50%	100%	20	3 min.	4 min.	Settles to bottom.	10	80-02	Looks good. Second best in this series. Warrants Field Testing.
9	90%	100%	30	5 min.	10 min.	Settles to bottom	10	80-02	Good. Best powder tested in this series. Warrants Field Testing.

(1) Wet screened with tap water.
(2) DDT powder added to 100 ml. of water in bottle to make $2\frac{1}{2}$% DDT suspension.
(3) Sprayed from a four gallon, hand-operated, air pressure sprayer.
(4) Suspension consisted of large aggregates which were not thoroughly wet.

a $2\frac{1}{2}$ percent DDT suspension. The wettability was evaluated by counting the number of shakes required to wet the powder. The shaking was done by hand using a back and forth motion through a distance of about 15 inches at a rate of about 120 complete oscillations per minute. To evaluate the stability factor, each powder was shaken 30 times, and the time for a noticeable amount of the powder to settle out was taken as the length of time such a suspension was stable. Each suspension was allowed to settle for one half hour, and was then reshaken 30 times. The time for a noticable amount of the powder to settle out was again taken. Except for No. 7, all of the powders were equally or more stable on reshaking than they were with the initial shaking. After each suspension had settled, the flocculation of particles was observed. Generally most of the powders settled to the bottom, but Nos. 2 and 3 separated and some particles went to the top of the water and some to the bottom. The same separation was observed on No. 7 with reshaking after standing.

The minimum pressure and nozzle opening required to spray each suspension satisfactorily was determined by spraying a $2\frac{1}{2}$ percent DDT suspension from a four gallon hand-operated air-pressure sprayer. During the spraying, the operator agitated the suspension as much as possible. The first two digits refer to angle of spray and last two digits refer to discharge rate, in tenths of a gallon per minute. Thus, 80-02 nozzle means 80° spray angle and discharge rate of 0.2 gal/min. Essentially same results were obtained with 50° and 65° nozzles as with the 80° nozzles.

None of the water wettable DDT powders tested were very good and all leave much to be desired in obtaining a stable suspension of DDT in water. Of the powders tested and reported in table 2, No. 8 and No. 9 will work satisfactorily for application from a hand sprayer, provided the suspension is agitated somewhat. The water wettable DDT powders can be sprayed more easily from power equipment because of the better agitation involved.

In the case of technical DDT such as is used for making xylene or kerosene solutions and emulsions, rather obvious differences in physical properties are of little practical importance. However, water-dispersible DDT powders obtained from different sources may appear similar but their properties may be so different that one may be satisfactory for use in hand sprayers whereas another similar appearing product may be unsatisfactory.

Recovery of DDT from the Exterior of Wild Rats

An attempt has been made to determine how much DDT rats pick up in running through DDT dusted areas. Two establishments, a poultry house and a cafe, were dusted moderately with 10 percent DDT dust. The poultry house was a two story building with a basement, of which the top floor and basement were dusted. The cafe was a three story building with a basement, all of which was dusted.

Snap traps, which killed the rats instantly, were used to collect the rats. This type of trap prevented the rat from thrashing around and losing any DDT it had picked up. The rats were taken out of the traps at the establishments, and put directly into clean beakers, which were brought back to the laboratory.

Each rat was soaked thoroughly in approximately 450 milliliters of benzene to dissolve the DDT from the exterior part of the rat. The solution was transferred to a 500 milliliter volumetric flask and the dirt, hair, etc., were allowed to settle to the bottom. Then aliquot portions were taken for analysis. Previously, a known amount of DDT had been placed on a laboratory rat, and 100 percent of the DDT applied was recovered.

In general, the rats collected from the poultry house were smaller rats and less DDT was recovered from these rats than the rats collected from the cafe. It is noted that the rats from the poultry house, near the end of the trapping period, showed less DDT recovered. This might be explained by the fact that because of the nature of the establishment, it was necessary to frequently sweep and clean the floors, thus removing considerable of the DDT applied. In the cafe, large rats were collected and considerable DDT was recovered from these. Also, near the end of the trapping period, more DDT was recovered than at the start of the trapping period, indicating the rat is likely to pick up more DDT the longer it is exposed to DDT dusted areas.

Check analyses on rats collected from a non-DDT dusted area showed only a small blank. The results are summarized in table 19.

Table 19. Recovery of DDT from the exterior of wild rats collected in DDT dusted areas.

DATE		WEIGHT OF RATS	DDT RECOVERED
DDT DUSTED	RATS COLLECTED		
	POULTRY HOUSE		
May 27, 1946	May 28, 1946	ADULTS	4.79 mg.
	May 28, 1946	NO	2.62 mg.
	May 28, 1946	WEIGHT	6.71 mg.
	May 28, 1946	TAKEN	4.77 mg.
	June 4, 1946	86 gm.	2.17 mg.
	June 5, 1946	59 gm.	1.39 mg.
	June 5, 1946	140 gm.	1.69 mg.
	June 5, 1946	67 gm.	2.72 mg.
	June 5, 1946	61 gm.	1.24 mg.
	June 6, 1946	57 gm.	0.44 mg.
	CAFE		
May 31, 1946	June 4, 1946	357 gm.	7.77 mg.
	June 4, 1946	102 gm.	0.78 mg.
	June 5, 1946	329 gm.	5.85 mg.
	June 5, 1946	250 gm.	13.60 mg.
	June 6, 1946	383 gm.	11.10 mg.
	June 6, 1946	513 gm.	5.21 mg.
	June 11, 1946	510 gm.	7.02 mg.
	June 11, 1946	432 gm.	47.80 mg.
	June 11, 1946	515 gm.	22.15 mg.
	June 11, 1946	393 gm.	10.41 mg.
	FARM		
NON DUSTED CONTROLS	June 4, 1946	341 gm.	0.06 mg.
	June 4, 1946	57 gm.	0.04 mg.

RODENT AND ECTO-PARASITE CONTROL BRANCH

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Investigations with 10 percent DDT dust.

The study of the effectiveness of 10 percent DDT dust for the control of rat fleas, which was begun in May 1945, was concluded after being carried on for slightly more than one year. As has been reported previously, effective control from a single treatment lasted for a period of approximately four months, or from June 1 to October 1. After that time the normal population of *Xenopsylla cheopis* in check stations became too low to warrant further conclusions concerning the effectiveness of control.

It was desired to learn if any residual effects of treatment could be detected in the treated establishments when the flea population began to build up the following season. For this reason the study was continued into June of 1946 when the normal *X. cheopis* population was again approaching a seasonal peak of abundance. The index of *X. cheopis* reached 7.7 per rat in untreated establishments on June 8, and in treated establishments on June 18. It was concluded that no practical degree of residual control remained in those premises which had been treated one year before.

ANTU-DDT Investigations.

Data gathered in Norfolk and Portsmouth, Va., concerning the use of 20 percent ANTU-10 percent DDT dust for the control of Norway rats and rat fleas is presented in table 20. This dust was applied to rat runs and blown into rat harborage areas and burrows where it could be contacted by rats. The rat population encountered in the above mentioned two cities during the study consisted, as far as could be determined, entirely of Norway rats. No evidence was observed of other species of rats existing in any of the establishments used in the study.

Table 20. Reduction in Norway rat population following use of 20 percent ANTU-10 percent DDT in Norfolk and Portsmouth, Va. - April 24 - May 11, 1946.

Type Establishment	Rats Recovered after ANTU (1)	Number of Odors (2)	Rats killed by other means following ANTU (3)	Estimated rats remaining after all treatments (4)	Degree of Reduction of rat population (5)		Remarks
					From	To	
1. Small Grocery	3	0	0	1-5	Moderate	Light	Tracks of about four rats in ANTU on day following treatment. All rats eliminated from store proper by ANTU. Damage to merchandise ceased after ANTU. Remaining rats, probably invaders, all entering through one hole in unoccupied rear room on 5th day following treatment.
2. Two-story Wholesale Poultry Abattoir	3	1	5	3	Heavy	Light	Large two-story brick establishment with abundant harborage in double floors in which poisoned rats could die. ANTU well tracked on day following treatment. Management reported noticeable reduction in abundance of rats following ANTU.
3. Three-story Meat Packing Company	10	1	11 + 4 mice	1-5	Heavy	Light	Three-story brick building with abundant harborage in double floors and walls of coolers in which rats could die. ANTU well tracked. Management reports marked reduction in number of rats. 1080 used three times.

Table 20. Reduction in Norway rat population following use of 20 percent ANTU-10 percent DDT in Norfolk and Portsmouth, Va. - April 24 - May 11, 1946. (continued)

Type Establishment	Rats Recovered after ANTU (1)	Number of Odors (2)	Rats killed by other means following ANTU (3)	Estimated rats remaining after all treatments (4)	Degree of Reduction of rat population (5) From	To	Remarks
4. Small Grocery	3	1	2	4	Heavy	Light	Many tracks in ANTU. Damage to merchandise usually heavy. Damage ceased for two nights after ANTU. Slight damage on 3rd night. None on fourth night. Slight on fifth night. Neighborhood rats have easy access into store. Definite reduction in amount of damage after ANTU. Two to three cups of 1080 touched. Invasion likely. No longer see rats.
5. Small Cafe and Confectionary	2	1	3 + 1 mouse	1	Moderate	Light	Many tracks in ANTU. Abundant harborage in walls and under floors. Easy access for neighborhood rats. Invasion likely.
6. Moderate Sized Grocery	3	1	3	0	Heavy	Light	Many tracks in ANTU. Abundant harborage under floor After ANTU, damage ceased in contrast to usually heavy damage before. Management well satisfied. Immediate invasion of another group of rats not likely because access is not easy.

Table 20. Reduction in Norway rat population following use of 20 percent ANTU-10 percent DDT in Norfolk and Portsmouth, Va. - April 24 - May 11, 1946. (continued)

Type Establishment	Rats Recovered after ANTU (1)	Number of Odors (2)	Rats killed by other means following ANTU (3)	Estimated rats remaining after all treatments (4)	Degree of Reduction of rat population (5)		Remarks
					From	To	
7. Small Cafe	0	1	1	2	Light	Light	Tracks of two rats in ANTU. No new tracks after five nights. One rat seen on fifth day. One rat killed after 1080. Ready access from outside. Rats subsequent to ANTU may have been invaders.
8. Small Cafe	1	1	2	1	Moderate	Light	ANTU well tracked.
9. Large Grocery and Delicatessen	31	3	25 + 2 mice	8 ±	Heavy	Light	ANTU well tracked. After ANTU nightly damage became negligible. ANTU not used in sales department of store, and used sparingly in delicatessen on second floor. Rat infestations remained in those parts of store until 1080 was used.
10. Small Grocery	1	3	6	1-5	Heavy	Light	Many tracks in ANTU. Damage reduced from estimated $5.00 nightly to neglibible. No longer see rats. Odor reported from beneath floor of home joined to rear of store. Much harborage available and ready access from outside. Invasion by neighborhood rats likely.

Table 20. Reduction in Norway rat population following use of 20 percent ANTU-10 percent DDT in Norfolk and Portsmouth, Va. - April 24 - May 11, 1946. (continued)

Type Establishment	Rats Recovered after ANTU (1)	Number of Odors (2)	Rats killed by other means following ANTU (3)	Estimated rats remaining after all treatments (4)	Degree of Reduction of rat population (5)		Remarks
					From	To	
11. Small Grocery	0	0	1 *	Few	Moderate	Light	ANTU well tracked. 1080 not used because rear storage area where infestation existed was open to the outside, presenting a danger to neighborhood cats and dogs.
12. Small Grocery	0	0	1	3	Moderate	Light	Many tracks in ANTU at four holes. No damage to merchandise following ANTU in contrast to damage usually expected. Rats have ready access from outside and invasion is likely.
13. Large Grocery and Wholesale Delicatessen	19 +3 mice	0	4	9 ±	Heavy	Light	ANTU well tracked. Rats eliminated from front store part. Several holes in ceiling of delicatessen part not treated. Remaining rats were in delicatessen.
Totals	76	13	64	34-46			

(1) All rats encountered were Rattus norvegicus.
(2) Each isolated odor is considered as one dead rat.
(3) Trapping 1080, etc.
(4) Based on observation of tracks in tracking dust.
(5) Based on pre- and post-ANTU estimates.
* Not counted in total summation.

It should be noted that no figures representing control are given on the table. Rather, the terms "heavy", "moderate" and "light" are used to indicate a comparison between pre- and post-treatment rat populations. It has become necessary to use these indefinite terms because the number of rats recovered following the use of ANTU-DDT frequently represents only a small part of the total number killed. This is apparent when it is realized that rats poisoned by ANTU do not die rapidly, but usually live from 18 to 30 hours after contact. This length of time permits rats to seek their habitual harborage which may be a considerable distance from the site of dusting.

Effective control depends largely on thorough treatment of all rat runs, burrows and harborage areas. It has been observed that where this has not been possible, rats using these runs, burrows and harborages have survived.

As in other tests, the degree of initial control achieved against rat fleas in the Norfolk and Portsmouth test was satisfactory. Data indicate that ninety-eight percent reduction resulted within four to five days following treatment. The pre-treatment rat flea index in the two cities was relatively low, being 1.5 for X. cheopis and 3.7 for all rat fleas. The determination of these indices centered around April 27.

Investigations with other Rodenticides.

Field tests have been undertaken with a rodenticide of German origin, 2 chloro - 4 - dimethylamino - 6 - methylpyrimidine, commonly known as "castrix". This rodenticide was submitted for testing by the U. S. Fish and Wildlife Service. The rodenticide was used in slightly acidified aqueous solution, at the rate of 1/2 oz. per gallon in three business establishments infested with Norway rats. No apparent kill resulted. Initial tests with caged rats indicate that the solution is not readily accepted. It has been suggested by the Fish and Wildlife Service that the sample submitted for field testing was not of satisfactory purity.

Field Testing of the Atlas Rodent Feeding Station.

Five rodent feeding stations, developed for dispensing 1080 solution, were submitted by a manufacturer for field testing. These stations are black metal boxes 12 inches long, 4 1/2 inches wide and 4 inches deep. A cover, hinged on the narrow dimension, may be locked. An opening 2 1/2 x 2 1/2 inches is provided at each end of the station to provide means of ingress and egress for a rat, but too small to enable a cat, dog or child to obtain the 1080 inside.

The boxes are made to fasten to the wall along rat runs and two zinc trays are fasten in each station. A souffle cup is placed in each tray to contain 1080 solution. These stations are intended to provide a safe means of employing 1080 solution as a rodenticide.

Tests were conducted with the stations in two Norway rat infested establishments over a total of 22 days - 11 days in each establishment, and five boxes per establishment. No rats were recovered during this period. In one establishment at the conclusion of the test, two souffle cups containing 1080 solution were placed at the former site of each station. During the following six days a total of six Norway rats were found dead and a seventh was killed when found suffering a typical 1080 convulsion.

While the stations were in position tracking dust was used to indicate the reactions of the rats to the stations. Only one rat was known to go through a station. Another entered partially but withdrew. Rats repeatedly ran over the top of a third and readily approached others, but did not enter.

Although the above described rodent feeding stations undoubtedly provide safety in using 1080 solution, their usefulness in their present form for the control of Norway rats is seriously doubted.

Factors which contribute to the failure of this device are as follows:

1. Norway rats do not seem willing to enter.
2. The stations cannot be used at many sites where it would be desirable to set out 1080 solution because:
    (a) They are too large (4" x 12" x 4 1/2")
    (b) Their use is further restricted by the manner in which the cover is hinged. Considerable space (15") is required vertically in order to service them.
    (c) The necessity of lifting this cover makes it useless to place the box against swollen, bulged or otherwise non-verticle walls where it may be desired to place 1080. Walls in this condition are of frequent occurence in older rat infested buildings.
    (d) They cannot be conveniently fastened against concrete, brick, or metal walls.

Rat Proofing Investigations.

Rat proofing investigations have been undertaken which incorporate the following objectives:

1. To determine the effectiveness of newly developed building materials for rat-proof construction.
2. To investigate the habits of rats in relation to rat-proofing operations, especially the factors associated with gnawing and nesting.
3. To develop measures by which building materials which are not considered rat-proof, may be protected against penetration by rats.

At the present time the species of rat being used is *Rattus rattus alexandrinus*. This species, being a ship rat, was chosen for initial test because of the current interest in materials used in ship construction. It is hoped that tests can be undertaken in the near future using *Rattus norvegicus* also.

After testing various methods and devices which might be used in carrying on the investigations, a box type cage (figure 3) was developed which seems to be satisfactory to use in conducting the tests. As the work progresses, further modification will undoubtedly be necessary. The dimensions of the cage are 12" x 28" with a height of 9". The floor is of metal and the sides are lined with 1/2" hardware cloth. The top is constructed of hard-

LEGEND:
A. Panel in slot.
B. Water.
C. Food
D. Rat on this side of panel during test.
E. Nesting box.
F. Exit

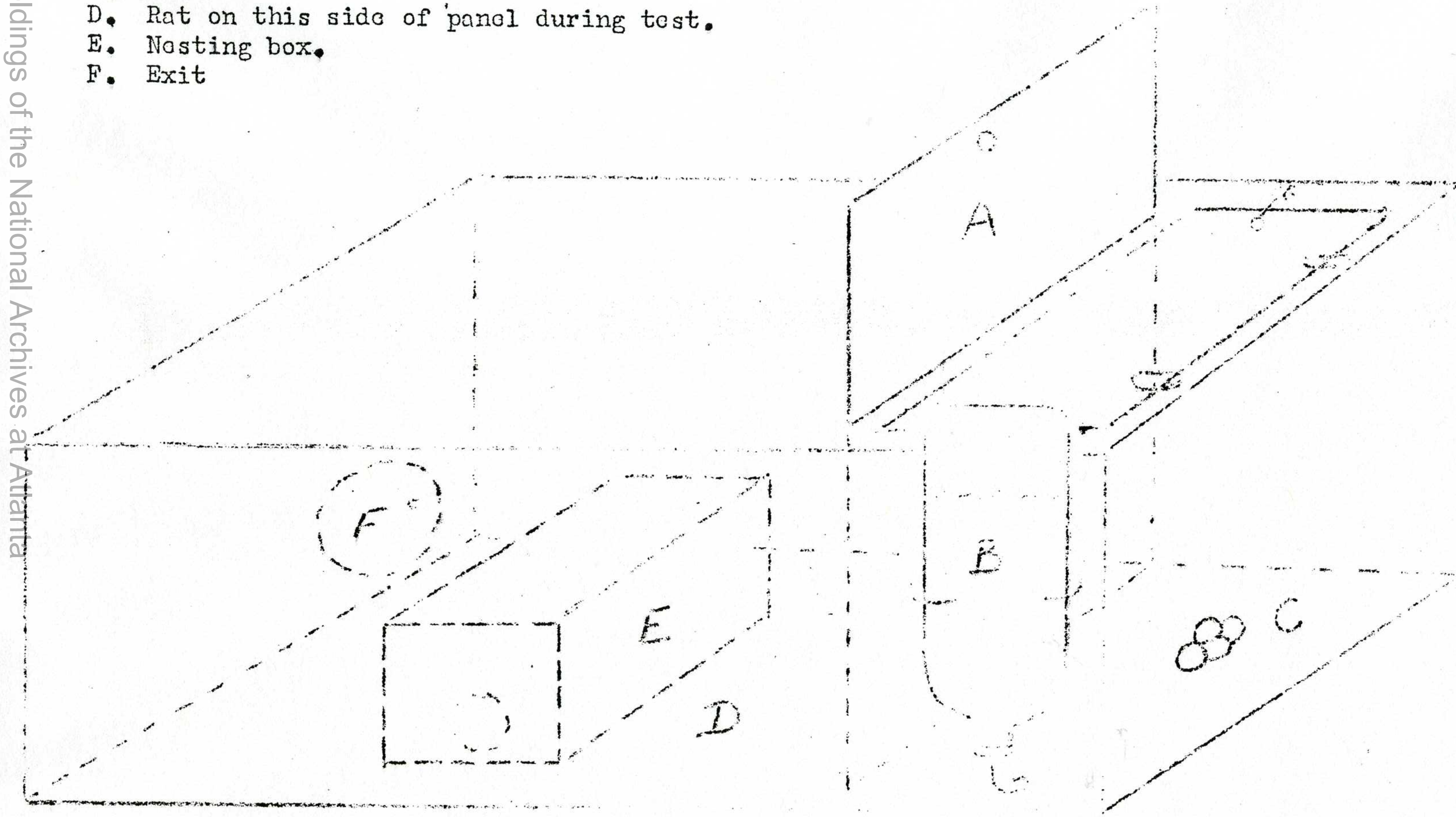

Figure 3. Rat cage for testing material panels.

ware cloth. and is divided into two parts. One is stationary while the other is hinged. A space exists between the two parts for the admittance of a panel of the material to be tested.

A single rat is confined in each cage. Food consists of Purina Laboratory Chow in the form of checkers.

A number of methods are being tried to stimulate the rats to gnaw. Each rat is confined to one part of the cage by a test panel which is set in place each night. In order to get water the rat must chew through the panel.

In addition food is being used as a stimulus. It has been determined that the test rats will consume on an average of about 16 grams of Purina Laboratory Chow in 24 hours. The rats are accordingly fed only about 10 grams per test day. Before the panels are placed each evening, all uneaten food is removed. However, food is placed on the opposite side of the panel along with water which the rat can get if it chews through the panel. In order to keep the animals in normal strength they are given an adequate supply of food on three nights over the week ends. A variation of this technic is to offer some particularly desirable piece of food, such as musk melon, as an incentive for chewing through the panel.

An attempt now is being made to develop in the rats a conditioned response. Wood panels with a small auger hole in the lower right corner were presented. A piece of musk melon was offered as a reward for enlarging the hole. The hungry rats responded readily. These panels will be kept in place during the daytime. The rats will get all its water and food by passing through this hole. At night the wood panel will be replaced by a test panel without a hole. This procedure has not been carried to the point where results can be given.

After observing that rats have a tendency to gnaw around the top of the boxes, a cage was constructed in an attempt to take advantage of this tendency. A cage of 1/2" hardware cloth but with no bottom was placed on top of the present cage, forming a two story cage. A part of the hardward cloth forming the top of the lower cage was removed in such a manner that a panel to be tested could be slid in on a horizontal plane. The rat in the lower cage can see through the hardware cloth on three sides of the panel. If it should succeed in gnawing through, it only escapes into the upper cage.

Some materials, such as thick cork insulation faced with a more durable type of building material, are to be tested. It is desired to learn if rats will enter this for nesting.

To test this material a cage 18" x 24" and 15" high was constructed with the insulating material to be tested set in a metal case facing on the back of the cage. The protective facing over the insulation may be broken to simulate a normal type of injury. A pair of adult male and female roof rats are placed in the cage.

Investigations also are under way to determine what physical conditions of the panels are necessary before rats will attack them. Panels which are placed flush with the floor of the test cages seem to be untouched unless such panels have dents, holes or scuffed places which provide a point of attack. Panels which are raised 1/8" from bottom seem to be less readily attacked than those which are raised 1/4". A gnawing edge, point of attack, or some other stimulus seems to be necessary before the rats will chew readily.

Of the materials being tested the following have been penetrated to date under the indicated conditions:

	Materials	Point of Attack Presented
1.	1/4" 3 ply wood	3/8" auger holes
2.	1/4" 3 ply wood	3/16" slot at bottom edge

Materials	Point of Attack Presented
3. 3/8" Gypsum board with calsomined surface	No opening presented. Calsomined surface toward rat.
4. Same as No. 3	1/4" slot along bottom edge
5. 1/2" Insul brick	1/4" slot along bottom
6. Same as No. 5	Rough edge on bottom
7. 1/4" 3 ply wood with 1/8" asphalt sheet	1/4" slot at bottom
8. 1/4" Masonite	1/4" slot at bottom
9. 3/8" Marine sheathing, plain finish.	Placed tight to bottom
10. Composition board, plaster surface backed by metal lath, 1" of fiber and sheet of iron. Total 1 3/8" thick.	Placed 1/4" from bottom. Plaster surface gnawed through. Some fiber pulled out. Metal lath prevented complete penetration.

DDT as a Rat Poison.

Although DDT is known to be toxic to rats, its general use in rat control has appeared impractical. However, since DDT is being employed widely in rat infested establishments for flea control, the question of its economical use as a rat poison again has arisen. Laboratory work is underway and will be continued as a basis for follow-up field investigations. Wild Norway rats have been exposed to DDT under conditions similar to, but somewhat more drastic than, the conditions encountered after the usual application of DDT in flea control. Of eight rats exposed to 10 percent DDT, the concentration currently in use for flea control, only one died. Of eighteen rats exposed to technical grade DDT, fifteen died after one to three exposures. Data will be acquired for intermediate concentrations and under different conditions more closely simulating field conditions.

Gammexane as Miticide.

Preliminary laboratory experiments indicate that hexachlorocyclohexane (Gammexane) has miticidal value. Field tests are inconclusive due to an insufficiency of mites at this season.

APPROVED:

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22 July 1946

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